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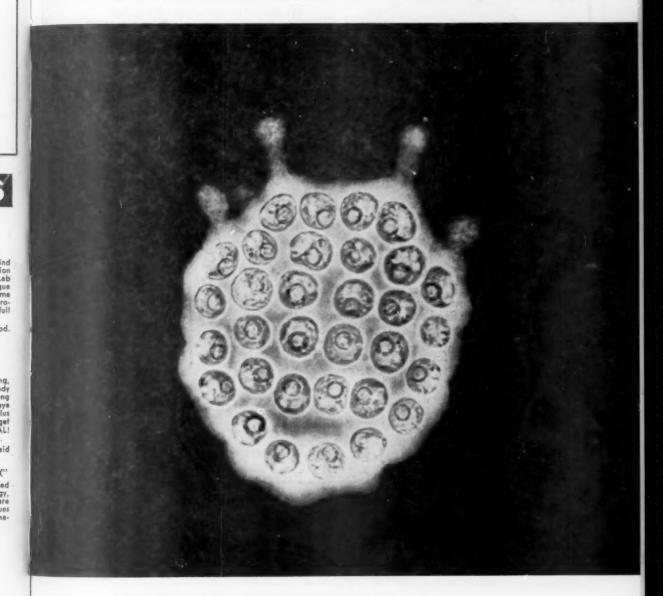
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The Experimental Approach Using The Microscope

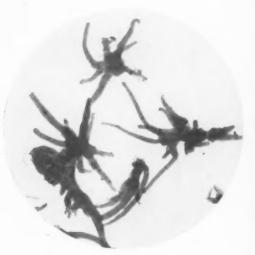
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Table of Contents

Robert W. Hoshaw	
AV News	499
Richard Fox	
Presenting—The Cellular Slime Molds	501
Summer Graduate Courses in Botany	505
AIBS Visiting Biologists Program	505
Geographic School Bulletin	505
Effects of Radiation on Germination Patterns of Spores of Certain Mosses and Ferns	506
"Vistas of Science"	512
Post-Doctoral Fellowships	512
Local Science Meeting	512
Women in Biology Leaflet	512
Root Tip Cell Squashes for the Study of Cell and Chromosome Morphology	513
NABT Teacher Award Program	515
Experimenting With Planaria John Gabriel Navarra and Donald R. Cicero	516
Have You Used Phluoroglucin Solution?	518
Wayne E. Manning	
NSF Graduate Fellowships	519
John Hays Fellows	519
AIBS Grant	519
Experimental Approach to the Study of	F20
Fresh Water Organisms	520
Aerial Spraying	522
Polarization of Cells and Crystals	523
Ted Stopyra	023
Dissection of the Common House Fly	524
Book Reviews	
Index for Volume 23	535

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Sexual Cycles Of Three Green Algae For Laboratory Study

• ROBERT W. HOSHAW, Department of Botany The University of Arizona, Tucson

Teachers define biology as the study of living organisms and then proceed needlessly to use preserved materials and permanent slides. On the other hand, it often is difficult and time-consuming to locate the desired organisms at the proper time. Fresh-water algae are excellent living material for biology teaching and are always readily available for classroom use.

The Culture Collection of Algae at Indiana University, organized and maintained by Dr. Richard C. Starr (1960), consists of over 900 cultures for purposes of research and teaching. These cultures can be obtained by high school and college teachers alike and provide ideal material for projects as well as classroom study. Each request should include \$1.00 per culture for the costs of handling and postage.

Several species in the Culture Collection undergo sexual reproduction when handled by the proper techniques. Thus, it is possible to study many of the stages in sexual life cycles under the conditions and with the equipment found in the conventional biology laboratory. In addition to showing the distinctive features of the life cycle of an alga, species can be selected to demonstrate variations in the sexual process. Cells of Chlamydomonas and Cosmarium both produce isogametes, but there is very little similarity in the method of gamete formation and gamete fusion. The experimental techniques used to handle three green algal genera are described in detail here to aid both teachers and students in the study of sexual cycles with the use of living material.

Terminology

The following groups of definitions will be helpful in understanding the nature of the cultures and the major events of the sexual process:

Clonal Culture: A group of organisms which have arisen from a single individual.

Pure Culture: A clonal culture of an organism (alga) from which all other organisms of any kind are excluded. Unialgal Culture: A culture which contains a single species of algae but from which all other organisms have not been excluded.

Homothallism: The condition in which sexual reproduction occurs in clonal cultures.

Heterothallism: The condition in which sexual reproduction occurs only when cultures of opposite mating types are mixed.

Mating Types: Individuals of heterothallic species which lack sexual differentiation, but readily participate in sexual reproduction. Such individuals are commonly designated as plus and minus.

Isogamy: Sexual reproduction with the fusion of identical gametes called isogametes.

Anisogamy: Sexual reproduction with the fusion of flagellate gametes of unequal size (Synonymous with Heterogamy)

Oogamy: Sexual reproduction with the fusion of a small flagellate sperm formed in an antheridium with a large nonmotile egg formed in an oogonium.

Zygote: The product formed by the fusion of gametes.

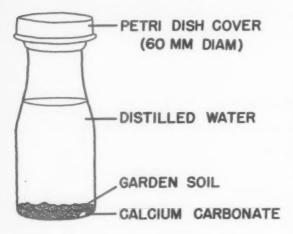
Zygospore: A thick-walled zygote produced initially by the fusion of isogametes or anisogametes.

Oospore: A thick-walled zygote produced initially during oogamy.

General Techniques

Soil-Water medium

To grow cultures of *Cosmarium* and *Oedogonium* requires the use of a medium unique to the culture of algae. Pringsheim's soilwater medium (Starr, 1960) is easy to prepare from readily available materials (Figure 1). About a teaspoonful of rich, unfertilized, garden soil is placed in a half-pint milk bottle along with a small pinch of calcium carbon-



ate. The bottle is then carefully filled threefourths full with distilled water and covered with a small petri dish cover. Bottles prepared in this way are steamed, but not autoclaved, for one hour on two consecutive days. This treatment will kill all algae in the medium and provide a bacterized medium for the growth and maintenance of unialgal cultures. To insure a clear medium free from excessive suspended particles use dry soil without too much clay.

Growth conditions

It is ideal to maintain and handle cultures in a lighted constant temperature room. However, only a few laboratories have such a facility. As long as cultures receive light of 200-400 ft-c intensity and are maintained at a temperature of 20-25 C, they should exhibit normal growth and activity. Cool white standard fluorescent tubes provide a suitable light source when cultures are placed within 8-10 in. of a single tube. If the lighting system is under the control of a clock device, it should be set to provide automatically 16 hr of light and 8 hr of darkness per 24-hr period. Other light cycles are satisfactory and Chlamydomonas can be grown under continuous light. Room temperature normally will be within the 20-25 C range.

Sterilization

Laboratories without an autoclave can use a pressure cooker, canner, or oven, including a kitchen oven. Sterilization of agar in a steam pressure device should proceed for 20 min under 15 lb of pressure. Glassware can be oven-sterilized at 160-170 C (320-338 F) for 2 hr. The unsealed cooker or canner is

suitable equipment in which to steam soilwater medium.

Chlamydomonas

The unicellular, motile, green alga, Chlamydomonas, is an ubiquitous alga with over 400 described species. It is commonly discussed in elementary textbooks in biology, botany, and zoology, and taxonomically appears to have an uncertain status among the kingdoms of living things. Chlamydomonas is characteristically biflagellate, uninucleate, with a single large chloroplast and a cellulosic cell wall. It has a simple sexual cycle easily studied within the limits of time imposed by a conventional laboratory period. By the use of complementary mating types the sexual cycle can be readily controlled. The vegetative cells of Chlamydomonas are haploid and produce 2-8 daughter cells which function as gametes. Gametes of heterothallic species fuse quickly to form zygotes. Meiosis occurs during zygospore germination and the resultant zoosperes are actually new vegetative plants. (See Figure 2.)

A. Cultures of Chlamydomonas

The mating types of several heterothallic species of Chlamy domonas are available from the Culture Collection of Algae. These are pure cultures maintained in a palmelloid condition on agar slants. Those recommended for use are C. reinhardtii, C. eugametos and C. moewusii. If only a single species is used during a laboratory period, C. reinhardtii shows more life cycle stages in a brief period of time. In C. reinhardtii clumping and pairing of gametes begin immediately upon mixing the mating types and quadriflagellate zygotes are visible within a few minutes. Swimming pairs can be viewed for several hours in C. eugametos and C. moewusii. In these latter two species zygote formation requires 8-10 hr.

B. Growth of Cultures

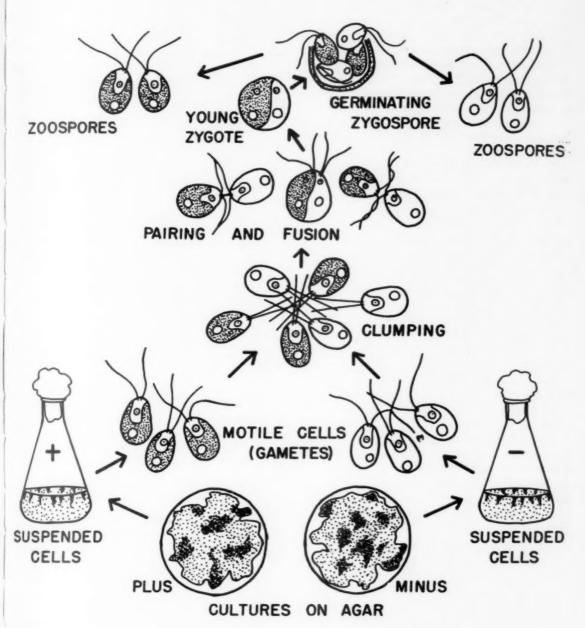
Culture of *Chlamy domonas* continue active growth on agar slants for 2-3 months without transfer. Five to 7 days before demonstrating sexuality, cells from the agar slants should be used to produce fresh agar cultures in petri dishes. The fresh agar cultures are prepared as follows:

1. Use 2 sterile petri dishes for each species. Half fill each dish with Bristol's agar or soil for 2 the

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SEXUAL CYCLE OF CHLAMYDOMONAS



soil extract agar. See Materials for agar formulae.

2. Place 6 or 7 drops of sterile water on the agar surface of each petri dish.

3. Transfer with a sterile bacteriological loop a generous quantity of green inoculum into the sterile water. Inoculate one plate

with the plus strain and one with the minus strain.

Mix the inoculum with the water and spread the suspension over the agar surface.

5. Invert the inoculated plates and place under a light of 200-400 ft-c at 20-25 C.

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C. Preparation of Gamete Suspensions

In 5 to 7 days a vigorous green growth of cells will appear on the agar surface of each plate. The preparation for the mating reaction requires the suspension of the cells in a liquid medium. The following procedure is used to prepare suspensions:

1. Wash the cells of each mating type from the agar surface with 20-30 ml of sterile distilled water the afternoon before the dem-

onstration of sexuality is scheduled.

2. Place each suspension in a 125-ml sterile Erlenmeyer flask and illuminate for 4-6 hr at 200-400 ft-c.

3. Cover the flasks and leave in the dark

overnight.

4. Return the suspensions to the light 2 hr before mixing them for zygote production.

D. Demonstration of Sexuality

If cells of the two mating types are mixed, the sexual response is immediate. This is first evidenced by clumps of cells, later by pairs of cells and finally by the fusion of cells to form zygotes. The details of the response vary with each species. The following procedure is used to demonstrate various stages of sexuality:

1. Place a drop of each mating type about half an inch apart on a clean glass slide.

- 2. Mix the two drops with a needle and note the clumping of cells (gametes). This should be done in an open drop without a cover glass. The clumping reaction takes place immediately after the 2 drops are mixed and within 2-3 min numerous pairs can be observed.
- Variations in the sexual response are as follows:
 - a. C. reinbardtii—the sexual response is rapid with clumping, pairing and fusion of gametes frequently occurring within 3-5 min. Pairing occurs first at the tips of the flagella, followed by complete agglutination of the flagella. Then the 2 protoplasts slip out of their respective cell walls; the flagella lose their attraction and gametic fusion ensues.

b. C. eugametos and C. moewusii—clumping and pairing occur rapidly in these species, but gametic fusion may not occur until 8-10 hr after mixing. The pairs are joined at their anterior ends and swim about

rapidly. Gametic fusion, when it occurs, is similar to C. reinhardtii.

4. After 5-10 min, add a drop of iodine solution to the slide with the mixed mating types of *C. reinhardtii*. This treatment kills the cells and makes the quadriflagellate zygotes easier to see.

Place a cover glass on the mount and examine under high power to observe quadriflagellate zygotes. Many biflagellate gametes

also will remain.

6. To obtain mature zygotes use sterile pipettes to place 4 drops of each mating type on the surface of an agar plate. Mix the suspended cells and spread the mixture over the surface of the agar plate with a sterile bac-

teriological loop.

7. Keep the zygotes which form on the agar in the light for 7 days and then store them in a dark place such as a box or drawer. The zygotes will enlarge to 4 or 5 times their original diameter and devolp a conspicuous thick wall as they become mature zygospores. When the plate is examined, it will be very easy to distinguish zygospores from unmated vegetative cells. DO NOT expect to see black, thick-walled, spiny zygospores such as are frequently pictured in textbooks.

E. Zygospore Germination

Zygospores matured on agar for 3 weeks germinate and produce from 4-32 zoospores which enlarge to become new vegetative cells. If the zygospores are allowed to dry on the agar surface, they remain viable for several years. Zygospore germination is obtained as follows:

1. Scrape off zygospores and unmated cells from the plate of mature zygospores with a sterile bacteriological loop. Place this material on the surface of a fresh agar plate in 5-7 drops of sterile distilled water and spread over the agar surface.

2. Invert this "fresh plate" for 30 sec over a petri dish bottom containing chloroform. This treatment will kill the vegetative cells.

5.

3. Put the plate in the light for 24-48 hr and then examine for germinating zygospores. Place a cover glass directly on the surface of the agar to view under high power.

4. Cover germinating zygospores with a drop of water to induce motility of the

zoospores.

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Below is a list of the materials required to demonstrate sexuality in *Chlamydomonas*. Sterile technique should be used at all times.

Cultures of Chlamydomonas (Culture Collection of Algae at Indiana University)

a. C. eugametos (Nos. 9 and 10)

b. C. moewusii (Nos. 96 and 97) c. C. reinhardtii (Nos. 89 and 90)

2. Media

a. Bristol's agar without iron or minor elements will suffice. It is prepared from 6 stock solutions, 200 ml in volume, each containing one of the following salts in the amount indicated:

 $\begin{array}{cccc} NaNO_3 & & 5.0g \\ CaCl_2 & & 0.5g \\ MgSO_4.7H_2O & & 1.5g \\ K_2HPO_4 & & 1.5g \\ KH_2PO_4 & & 3.5g \\ NaCl & & 0.5g \\ \end{array}$

Add 5 ml of each stock solution to 470 ml of distilled water along with 7.5g of powdered agar. Autoclave this mixture for 20 min at 15 lb pressure. Just before the warm agar solidifies pour into sterile petri dishes. Approximately 15 dishes can be prepared from 500 ml of agar.

b. Soil extract agar is prepared in 500 ml quantities as follows:

450 ml distilled water

5 ml of each of the 6 stock solutions described above

20 ml of supernatant from a soil-water bottle

7.5g of powdered agar

Autoclave for 20 min at 15 lb pressure.

3. Sterile petri dishes

a. bottoms-95 mm diameter; 15 mm depth b. covers-100 mm diameter; 12 mm depth

 Sterile pipettes—di SPo-pette, disposable capillary pipettes, 9-in. long (available from Scientific Products, 1210 Leon Place, Evanston, Illinois, and Aloe Company, 5655 Kingsbury, St. Louis 12, Missouri.)

5. Bacteriological loop

6. Chloroform

7. Sterile distilled water (if available, use glass-distilled or ion-exchanged water)

8. Tincture of iodine or iodine-potassium iodide reagent. Prepare iodine-potassium iodide reagent by dissolving 1g potassium iodide in 100 ml distilled water; then add 1g iodine.

Cosmarium

Desmids have been favorite objects of microscopic study for many years. In addition to their beauty, they are uniquely suited to demonstrate the conjugation process. While Spirogyra is most commonly used to show this process, the cells of a genus such as Cosmarium exhibit the details from gamete development to zygote formation in a much more spectacular way. Cosmarium is a unicellular desmid with a deep constriction between the semicells. Each semicell may contain 1-4 chloroplasts. The nucleus is situated in the isthmus. Both homothallic and heterothallic species of Cosmarium exist in nature and are easily cultured. When cells are placed under appropriate environmental conditions, they pair and become surrounded by a common gelatinous sheath just prior to conjugation. Each cell then breaks at the isthmus and the amoeboid movement of the protoplast (gamete) from each cell results in the formation of a zygote midway between the cells. The zygote enters a dormant state, but after 2 or 3 months germinates to produce 2 daughter cells which enlarge and become new vegetative cells. The cells of Cosmarium are haploid with meiosis occurring in the zygote. The discussion which follows outlines the techniques to induce the sexual cycle of two species, Cosmarium botrytis and C. turpinii. This latter species was earlier identified as C. botrytis var. subtumidum in papers by Starr (1954, 1955). (See Figure 3.)

A. Growth of Cultures

Cells of *C. botrytis* and *C. turpinii* grow well in soil-water medium. To obtain actively growing cultures handle cultures in the following way:

1. Transfer cells of recently acquired cultures into bottles of soil-water medium to increase the quantity of cells available for

mating.

2. Place the cultures in light of 200-400 ft-c at 20-25 C for 10-14 days or until a dense culture develops.

3. Remove the cultures to a shelf with light of 20-25 ft-c until ready for use.

B. Demonstration of Sexuality

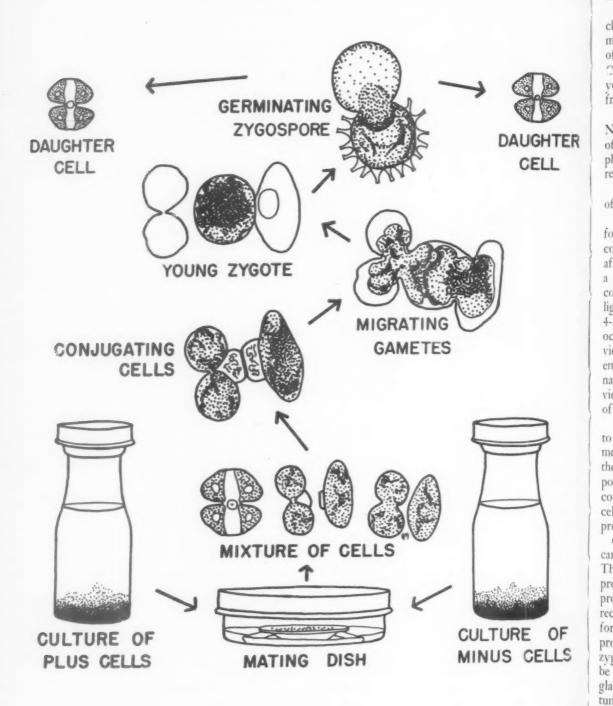
The best sexual response results from cells of actively growing cultures. There is little difference in the response of *C. botrytis*

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SEXUAL CYCLE OF COSMARIUM

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(homothallic) and *C. turpinii* (heterothallic) except in the latter species plus and minus strains must be mixed to induce conjugation. The demonstration of sexuality is carried out as follows:

1. Prepare a mating dish by filling the chemical watch glass half full of cells and medium of *C. botrytis* or by filling half full of a mixture of the plus and minus cells of *C. turpinii*. The cells may be from cultures you have grown or from material received from the Culture Collection.

2. Add 20 ml of a freshly prepared 5% NaHCO₃ solution to the petri dish portion of the mating dish. The increase in atmospheric carbon dioxide hastens the sexual response.

3. Cover the mating dish and place in light

of 200-400 ft-c at 25 C.

4. Check the cells for pairing and zygote formation at the end of 48 hr. In *C. botrytis* conjugation is greatest during the first hour after a culture returns to the light period of a 16-hr light-8-hr dark cycle. In *C. turpinii* conjugation is most prevalent 4-6 hr after the light cycle begins. It will probably require 4-5 days for numerous gametic fusions to occur. Cells pair in such a way that when viewed microscopically the elliptical-shaped end view of one cell appears adjacent to the narrow side view of the other cell. The best view of vegetative structure is the front view of unpaired cells.

5. For laboratory study, instruct students to mount on a clean glass slide 2 drops of medium and cells taken from the edge of the mixture and observe under low and high power. Place several small pieces of broken cover glass on the slide before adding the cells and cover glass. This will prevent undue

pressure on conjugating cells.

6. Instruct students to check paired cells carefully for incipient stages of conjugation. This is first evidenced by the formation of projections at the isthmus of each cell. Such projections form a tubular connection which receives the gametes at the time of zygote formation. Once gamete movement begins it proceeds continuously for 15-30 min until zygote formation is complete. Care should be taken to keep the mount moist. The cover glass may be sealed to the slide with petrolatum. If one student finds cells undergoing conjugation, there is sufficient time for others

to view the mount. It is not expected that all students will find conjugating cells in the limited time available for making observations. An excellent series of photomicrographs of sexual reproduction in *C. turpinii* appears in a paper by Starr (1954).

7. Note that many of the zygotes have developed into spiny-walled zygospores. Allow these zygospores to dry slowly in the watch glass for use later in zygospore germination.

C. Zygospore Germination

The zygospores of either *C. botrytis* or *C. turpinii* require 2-3 months of dormancy before they will germinate. To attempt germination cover dried zygospores with fresh soilwater medium and place in light of 200-400 ft-c at 20-25 C. Observe each day for signs of germination. Meiosis occurs in the zygospore and 2 daughter cells are produced. The details of zygospore germination are recorded photographically in a paper by Starr (1955).

D. Materials

The materials needed to demonstrate sexuality in *C. botrytis* and *C. turpinii* are listed below. While sterile technique is not used to handle these species, care must be taken to keep the cultures unialgal.

Cultures of Cosmarium (Culture Collection of Algae at Indiana University)
 a. C. botrytis (No. LB 953)
 b. C. turpinii (Nos. LB 733 and 852)

2. Soil-water medium (See General Tech-

niques)

3. Mating dish—prepare from a deep petri dish (20 mm), a chemical watch glass (65 mm) and a glass triangle. The triangle is used to support the watch glass and is made by bending 5 mm glass tubing.

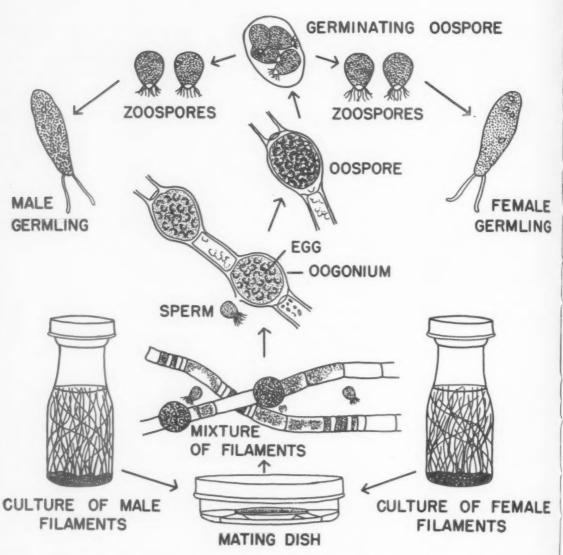
4. Sterile pipettes

5. Sodium bicarbonate (NaHCO₃), 5% solution

Oedogonium

Oedogonium cardiacum is a haploid, heterothallic, unbranched, filamentous alga. The species is macandrous and sexual reproduction is oogamous with the large oogonia and small antheridia produced on separate plants. If male and female filaments of an old culture in the vegetative condition are mixed, egg and sperm formation occur within 36-48

SEXUAL CYCLE OF OEDOGONIUM



hr. Each oogonium produces a single, large egg; antheridia, which occur in series of cells, form 2 sperm each. The sperm have a distinctive ring of flagella at the anterior end and swim rapidly in liquid medium. Gametic fusion occurs within the oogonium and the resultant zygote develops into an orange-colored oospore that has a long dormant period. In certain species of *Oedogonium* it has been shown that meiosis occurs in the oospore and germination produces 4 zoo-

spores, each of which develops into a new filament. Below is a description of methods to demonstrate several stages in the sexual cycle of *O. cardiacum*. (See Figure 4.)

A. Growth of Cultures

Filaments of *O. cardiacum* grow well in soil-water medium. Old cultures of this algawill remain healthy and can be induced to produce eggs and sperm over a period of several months. Sexually active cultures are grown as follows:

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and wh por 1. With a sterile pipette introduce male and female filaments into separate bottles of soil-water medium.

2. Place the cultures in light of 200-400 ftc at 20-25 C for 4-6 weeks or until a dense

culture develops.

3. Remove the cultures to a shelf where they will receive only dim light of 5-10 ft-c. These cultures should remain green and healthy for several months.

B. Demonstration of Sexuality

Old filaments which have ceased active growth begin to produce oogonia and antheridia in 36-48 hr if handled in the manner described below. The sexual structures develop when the male and female filaments are induced separately as well as when they are mixed. A mixed culture is considered here:

1. Prepare a mating dish by filling a chemical watch glass one-half full of the supernatant from fresh soil-water medium.

- 2. Use a sterile wire or glass hook made from a pipette to lift a mass of old filaments from each culture bottle and with sterile scissors cut to a $\frac{1}{2}-\frac{3}{4}$ in. length. It is also possible to use filaments received directly from the Culture Collection.
- 3. Place the masses of male and female filaments in the mating dish. With a sterile needle or pipette spread the filaments uniformly throughout the watch glass. Take special care to arrange filaments completely out to the edge of the medium.

4. Add 20 ml of a freshly prepared 5% NaHCO₃ solution to the petri dish portion of the metric dish

of the mating dish.

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5. Cover the mating dish and place in light of 200-400 ft-c at 25 C.

- 6. Check the filaments in the mating dish after 36-48 hr and continue to check periodically thereafter. Most fertilizations will have occurred 72-96 hr after the filaments are mixed.
- 7. For laboratory study, instruct students to mount several filaments and a drop or two of the surrounding medium on a clean glass slide for observation under both low and high power. Note carefully any sperm which are active in the vicinity of oogonial pores.
 - 8. To demonstrate mature zygotes (oo-

spores) mix filaments 7-10 days before a scheduled laboratory period. The orange oospores remain within the oogonial walls.

C. Oospore Germination

The newly formed oospores of Oedogonium frequently enter an extended dormant period. Oospores from one year's laboratory period should be kept for germination the following year. Oospores in the dried condition and others kept moist in the original mating medium can be stored. Five to 7 days before needed, cover the oospores with fresh soil-water medium and place in the light. Check daily for the appearance of zoospores. In Oedogonium meiosis occurs in the oospore.

D. Materials

The materials needed to demonstrate sexuality in *O. cardiacum* are listed below. While sterile technique is not used to handle this species, care must be taken to keep the cultures unialgal.

 Cultures of O. cardiacum (Nos. LB 39 and 40, Culture Collection of Algae at

Indiana University)

2. Soil-water medium (See General Techniques)

3. Soil-water supernatant

4. Mating dish (See Materials under Cosmarium)

5. Sterile pipettes

6. Sodium bicarbonate (NaHCO₃), 5% solution

7. Scissors

Discussion

The three organisms under discussion were selected because they can be grown and maintained with ease and show considerable variation in the details of the sexual process. It is suggested that Chlamydomonas be used to show a complete sexual cycle, including zygospore germination. Oedogonium is an ideal organism for demonstrating egg and sperm formation since both structures are large and the sperm swims rapidly in liquid medium. While conjugation involving the formation of a tubular connection is most frequently studied in Spirogyra, the conditions under which it occurs are much better defined for Cosmarium and gamete movement is rapid. However, in both Oedogonium and Cosmarium, oospore and zygospore germination, respectively, are somewhat difficult to control under laboratory conditions.

498

In an attempt to grow and control the activity of living organisms under laboratory conditions, it should be remembered that natural environmental conditions cannot be duplicated. However, there is a wide range of conditions under which organisms normally will react. In the foregoing discussion workable conditions have been defined within narrow limits, but other combinations are certainly possible and should be tried to suit the conditions of your own laboratory. A higher temperature, for example, may noticeably increase the growth of a culture and speed the sexual process while a high light intensity or direct sunlight may kill a culture.

Not all of the suggested equipment and materials will be found in every laboratory. In this regard "necessity is the mother of invention." Sterile technique commonly involves the use of an autoclave, but as mentioned earlier, a pressure cooker, canner, or an oven may serve as well. For steaming soilwater medium any container in which the bottles are surrounded by steam will suffice. Substitutions for various suggested types of glassware are just as possible. In the final analysis it is the study of the activity of the living organism which counts.

Acknowledgment

The author wishes to express his sincere appreciation to the Department of Botany at Indiana University for making facilities available for his work during the 1960-61 academic year while he was a National Science Foundation Science Faculty Fellow.

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Cosmarium

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lgae ulae ainaper Dr. of ton, STARR, R. C. 1954. Heterothallism in Cosmarium botrytis var. subtumidum.* Am. J. Botany 41: 601-607. (Contains an excellent series of time-lapse photomicrographs of the stages in sexual reproduction.)

STARR, R. C. 1955. Zygospore germination

in Cosmarium botrytis var. subtumidum.*
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*Cosmarium botrytis var. subtumidum was later identified as C. turpinii.



RICHARD FOX, Richwoods Community High School, Peoria Heights, Illinois

We hope many of you will come to the AV showings of film and filmstrips during the AAAS—NABT meetings in Denver this month. Several newly released films describing various techniques used in biology will be presented as well as films on such subjects as space biology, radiation biology, laboratory planning, and many others.

The meeting will be in the Empire Room of the Shirley-Savoy Hotel from 9 to 11:30 a.m. and from 2 to 4 p.m. on Friday, December 29, 1961. Why not stop in and meet your friends, enjoy these new releases, and let your wishes and desires for our AV column be known?

Encyclopedia Britannica Films has available a series of new biology films. The series is divided into the following units: Ecology, Plant Life, Animal Life, Physiology, and Heredity and Adaptive Change. Altogether the series consists of 28 films with more to be announced soon. The EBF program emphasizes the concepts of ecology and evolution throughout each series. A brochure of information is available.

Baboon Behavior (C-31 min.) A vivid description of the many facets of the baboon's behavioral patterns filmed in their natural habitat. Group shots as well as individual close-ups allow the viewer to see specific actions often missed by even the experienced observer. The social aspects

of the baboon's life is the major theme of the film. Such topics as grooming, protection, troop organization, and interrelations of the various age groups are supplemented by footage showing the eating habits, sleeping habits, and sexual behavior. Department of Visual Communication, University of California.

The Life and Death of a Cell (C-26 min.) Illustrates how the cell embodies all the functions and properties common to living things. The first section of the film, using a combination of animation and microcinematography, shows habitat, morphological details, digestion, and egestion of the ameba. The last portion of the film permits one to see by microsurgery the importance of the nucleus. This is followed by a sequence showing cell division. A section on the effects of light, touch, other organisms, and pH is presented. The final section describes how the inability of the cell to adjust to its environment leads eventually to its death. Department of Visual Communication, University of California.

The World Within (C-31 min.) This is a very good film on parasitology. Clear, concise definitions of parasitism, commensalism, mutualism, and saprophytic relationships are substantiated by various examples of living parasites and their hosts. Department of Visual Communication, University of California.

The Cave Community (C-13 min.) Relates how the dark and unchanging environment permits the biologists to study the physical features of the plants and animals encountered. Questions

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lamand of aggressive and regressive evolution, food webs, and physiological adjustments are brought out into the open and reasons advanced for their being. EBF, 1150 Wilmette Ave., Wilmette, Illinois.

Succession—From Sand Dune to Forest (C-17 min.) Shows the step by step development of a sand dune area into a beech-maple forest. The action of several intermediate plants and animals of the succession is brought to the observer's eye by close-up action shots. EBF, 1150 Wilmette Ave., Wilmette, Illinois.

Beargrass Creek (C-26 min.) Describes the water pollution problem of several states along the Ohio River. These eight states have banded together to battle water pollution under a mutual Ohio River Valley Water Sanitation Commission. Industrial wastes, city problems, water sport problems, and problems of irate citizens are depicted, and methods of motivating community understanding are presented on a basic level, but it effectively gets the point across. State Health and Sanitation Departments, States of the ORVWSC.

Marshes of the Mississippi (C-13 min.) Explores the life of the marshlands along the Mississippi River near the Gulf of Mexico. Indicates the forces acting to build up the marshlands, modes of transportation in the region, the flora and fauna of the area, and the dependence of man upon the conservation and harvesting of the animals of the delta region. Avalon Daggett.

R.L.F.

How Nature Protects Animals (C-11 min.) Points out the struggle for survival that exists among animals; illustrates the means animals use to evade their enemies; and gives examples of protective devices, such as, ability to run rapidly, mimicry, coloration, armor, and secluded homes. EBF.

Partnerships Among Plants and Animals (C-11 min.) Uses a variety of examples to show the interdependence of plant groups, animal groups, and plant-animal groups. Coronet.

Secrets of the Ant and Insect World (C-12 min.) Explores through close-up photography the variety of ways various species of ants adapt to their environment and use it constructively. Walt Disney.

Secrets of the Bee World (C-13 min.) Follows the life cycle of the honey bee, as found outside the commercial hive, with attention to their duties in the hive and reproduction. Walt Disney.

Secrets of the Plant World (C-15 min.) Surveys, using time lapse photography, the means used by plants for adaptation and survival, placing emphasis on means of seed dispersal, seed germination, and plant growth. Walt Disney.

Secrets of the Underwater World (C-16 min.) Surveys some of the various species of animals living in fresh and salt water and the animals' means of adaptation. Walt Disney.

The Spruce Bog-An Essay in Ecology (C-22 min.) Shows the ecological evolution that takes place in the formation of a spruce forest from the open water stage through spruce bog to the final stage when the bog has been replaced by a spruce forest. McGraw-Hill.

George Vuke Indiana University SI

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Attend!

NABT meetings with AAAS in Denver, December 26-30, 1961.

NABT headquarters: Shirley-Savoy Hotel.

Presenting --- The Cellular Slime Molds

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· Arthur D. Feiro, Port Angeles Senior High School, Port Angeles, Washington

The cellular slime molds, free-living members of most soil communities, provide an always welcome, living addition to our high school laboratory. These members of the Acrasiales, particularly the family Dictyosteliaceae, are excellent for school laboratory use because of their simple maintenance and culture requirements as well as their ease of isolation. You may well be asking yourself, as I did, why if it is such an excellent laboratory addition have we not heard of it before? K. B. Raper (6), the leader in cellular slime mold research, answers this question by pointing out that if their abundance in nature were generally appreciated and their ease of maintenance understood they would appear far more often in the teaching laboratory. A discussion of the techniques for isolation and culturing, as well as their truly unique life history, will be my part to acquaint you with these eminently useful laboratory "tools."

Life Cycle

From an evolutionary standpoint the Acrasiales are believed to occupy a position near the divergence of the plant and animal kingdoms. As you will see, they do exhibit clearly delineated characteristics of both kingdoms.

The Acrasiales are often confused with other aggregation organisms, but the similarities are only superficial. The difficulties of classification are further compounded by the lack of an adequate key. Bonner (3) includes a partial key in his recent book on the cellular slime molds.

The problem of identification would at this point seem insurmountable. Fortunately this is not the case since once having seen a living representative or its picture they are easily identified. Aside from the fact that they are "slimy" they have little relationship with the most commonly confused aggregation organisms. Some important differences are presented in Table 1.

One of the most striking attributes of the Acrasiales is its display of a life cycle made up of several distinct stages. The degree to which these growth phases are differentiated is quite foreign to other groups of organisms.

During the vegetative or feeding phase the myxamoebae are free living and uninucleate. They multiply by binary fission, each daughter cell remaining uninucleate and independent of the other.

At a point when the food supply begins to diminish, clearly observable orientation of these free-living myxamoebae to a central area is noted. See Figure 1. This activity inaugurates the aggregation phase. Bonner (2)

Table 1. Differences among the Mycetoza*

Myxomycetales	Flagellated swarm cells Sexual reproduction True plasmodium	
Plasmodophorales	Bi-flagellate swarmer Sexual type reproduction Small haploid plasmodium	
Labyrinthales	Net-like projections utilized in amoeboid travel. Occasional crude aggregation forms	
Acrasiales	As described in article	

^{*}Adapted from materials presented in Bonner's recent book on the cellular slime molds. (3)

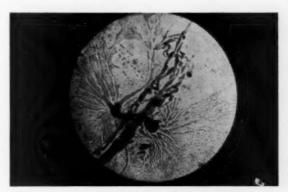


FIGURE 1. Two streaming aggregation bodies are seen in this microphotograph. The paths that resemble a river delta area are the avenues followed by the migrating myxamoebae. The darker masses toward which they are streaming will be the pseudoplasmodia. (50X)



FIGURE 2. The distinctive shape of the migrating pseudoplasmodia can be clearly seen in this photograph. This is the migrating form of *Dictyostelium discoideum*. (50X)

points out that the causative stimulus for this phase is a chemotactic attractant secreted by centrally located myxamoebae.

This central aggregation of cells, still maintaining their cellular integrity, form a "slug-like" mass. See Figure 2. This mass, now called the pseudoplasmodium, has raised itself vertically through the incorporation of additional aggregating myxamoebae. The pseudoplasmodium then falls to the media surface and migrates from the aggregation site.

The culmination phase now begins. It will result in the formation of a reproductive unit. The cells anteriorly located in the pseudoplasmodium begin to form a sorocarp. The posteriorly located cells form the spore bearing sori. The cells forming the stalk and the spores in the capsule are then covered with a cellulose secretion. Each of the many spores so encased contain a myxamoebae and upon being introduced to a proper environment will repeat this most interesting life cycle.

Culture and Isolation Methods

These organisms grow in conjunction with a bacterial host upon which they feed during their free living phase by phagocytosis. Their bacterial host range is quite wide (Raper, 4). The bacterial associates, vital to their culture, are easily obtained. *Escherichia coli* is most often used because of its ready availability,

'Pseudoplasmodium is used in lieu of the term plasmodium to describe the aggregation mass of *Dictyostelium* because it retains its cellular identity. In the myxomycetes this cellular individuality is lost. though any of a number of bacteria will serve as well. Some bacterial associates that have been found to support adequate growth of Acrasiales in our laboratory are listed in Table 2. A more complete list for *Dicty ostelium discoideum* is available in a study by Raper (4). The bacterial host used by my students is a species of *Lactobacillus* isolated from cottage cheese. We do, however, use *E. coli* for storage purposes.

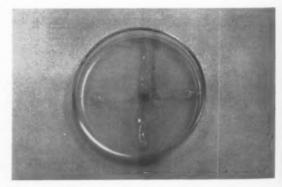
To insure against an excessive growth of bacteria, a *nutrient-poor* agar is employed. A simple hay infusion agar prepared according to the directions given here is one that is commonly used.

Hay Infusion Media (Suggested by Raper, 4)

- a. 35 gm of hay (any type is suitable).
- b. Add one liter of tap water.
- c. Pressure cook for one half hour at 10 lbs, pressure.
 - d. Filter and make up to one liter of liquid.
 - e. Add 0.2% K₂HPO₄.
 - f. Add 1.5% agar.
- g. Autoclave mixture at 15 lbs. pressure for twenty minutes.

The media is stored in test tubes under refrigeration. When needed it is liquefied and plated either in a petri dish or medicine bottles.

To insure ease of observation the bacteria should be streaked on the nutrient surface to allow their growth pattern to fashion a large



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FIGURE 3. This picture shows the growth pattern utilized in the isolation and cultivation of the cellular slime molds. In this particular photograph we are inoculating a soil particle at the juncture of the two growth lines. The bacterial associate in this case is *E. coli*.

Table 2. Cellular Slime Mold Growth With Selected Bacterial Associates*

Bacterial Host	D. mucoroides	D. discoideum
Pseudomonas aeruginosa	Good	Good
Bacillus polymyxa	Good	Slight
Bacillus megatherium	Slight	Poor
Bacillus my coides	Slight	Poor
Salmonella pullorum	Slight	None
Aerobacter aerogenes	None	None
Rhizobium sp. (Alder)**	Good	Good
Bacillus cereus	None	None
Lactobacillus sp.**	Good	Good
Bacillus subtilis	Poor	None
Escherichia coli	Good	Good
Serratia marcescens	Slight	Slight
Pseudomonas fluorescens	Slight	Slight
Staphylococcus albus***	None	None
Staphylococcus aureus***	Good	Slight
Alcaligenes faecalis	Good	Slight
Sarcina lutea	Good	Slight

"These are the partial results of work conducted in our laboratory.

**These two were isolated by my students from local sources.
***May be referred to as Micrococcus pyogenes (var. aureus) and Micrococcus pyogenes (var. albus).

X. See Figure 3. The bacteria are incubated at 37°C for two days. We then may introduce the spores of Dictyosteliaceae, or soil we are testing, at the juncture of the bacterial growth lines. See Figure 3.

To facilitate the inoculation of Dictyosteliaceae we use a standard inoculating loop. We also found an inoculating needle ground to a spatulate form to be excellent for picking up spores from the media surface. When the spore capsule falls to the media, usually some distance from the growing bacteria (see Figure 4), it is picked up with a sterile loop and planted as outlined before. By using spores isolated a short distance from the bacterial growth we minimize the possibility of bacterial contamination.

Our original cultures were obtained from a university source.² Subsequently we have been able to isolate them from soils in our town.

Isolations of common species of Dictyosteliaceae have been made quite frequently in the midwest by Raper and Thom (9), all helping to illustrate their abundance and wide dispersal in nature. We maintained our cultures at room temperature giving them no special care. Samples were taken from the same source for many weeks before it was invaded by unwanted molds or bacteria.

Some of my students have maintained cultures of *D. discoidium* and *D. mucoroides* in a refrigerator at about 10°C during the past two months. They have removed viable spores several times during this period.

In preparing cultures for storage it is wise to allow a growth period of some eight to ten days to elapse to assure adequate spore production. Normally refrigerated species of Dictyosteliaceae should be renewed every three months. Using a lyophil process suggested by Raper and Alexander (8) cultures have been kept viable for nine years. Details of this somewhat involved process are included in the above cited article.

Experimental Potential

The technique described for the isolation of the mold is an excellent group activity to initiate the laboratory phase of our work. This introductory activity allows a familiarization with the streaking technique and also the simple manipulations involved in inoculation.

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The author will be able to furnish cultures of *D. discoideum* or *D. mucoroides* if a test tube slant of nutrient agar is mailed along with provisions for return postage. These cultures travel quite well in the mails if the tube is packed in a mailing tube.

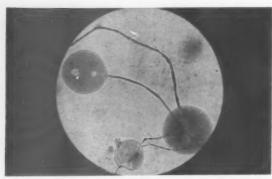


FIGURE 4. The spore head or sorus can be seen as it appears having recently fallen to the media surface some distance from the bacteria. The dark lines are the stalk or sorophore. Picking up these spore masses to inoculate a new culture is not difficult.

Seldom does one fail to turn up a representative of the Acrasiales, often *D. discoidium*. We have been most successful in isolating cellular slime molds from well cultivated and fertilized areas.

From this usually quite exciting beginning containing all the elements of a good mystery, enthusiasm runs quite high for this unusual organism. Ideas for individual or small group investigations can now be identified by your classes.

Because of the unique many-phase life cycle the Dictyosteliaceae lend themselves ideally to individual or small group investigations. This is particularly true in the study of physico-chemical affects on structural variations. Some groups might study the affects of heat and light on each of the growth phases. Exposing them to various radiation products should yield some interesting results. Since this organism has as its laboratory environment a petri dish the construction of experimental apparatus should know few boundaries.

Still the most popular with my students is the isolation aspect of the investigation. Reports concerning soil pH, flora found in the areas of mold isolation, plus many other variables, present excellent original research opportunities.

The preparation of nutrient-poor media from randomly assembled ingredients would be valuable particularly when it is coupled with an attempt to maintain the Dictyosteliaceae on a bacterial host which had failed to support growth previously. Investigations of a more sophisticated nature might begin with the growth of the cellular slime molds in conjunction with a pigmented bacterial host such as Serratia marcescens. Since this pigmentation will also color the slime molds a whole new area of manipulative and cutting experiments are revealed.

Transplanting pseudoplasmodial parts to study the origin of sorocarp formation is possible with this naked cell mass. Studying the results of wholesale removal of sections from the migration mass would prove quite interesting as well.

Observing the effects of bacterial metabolites on the pH of the substrate would prove revealing when compared with bacterial and

slime mold growth.

The experimental possibilities, as you can see, are limited only by the imagination of the teacher or student. With cultures of these delightful organisms we can witness many of the more elusive biological principles and functions we do so strive to inculcate in our students.

We can observe phagocytosis by an amoeboid cell, as well as a complete life cycle clearly demarked by distinct phases. Here is a rare opportunity to study the growing stage clearly separated from the morphogenetic phase. Specialization of hitherto unspecialized cells to form the pseudoplasmodium, the sorocarp with its basal disk and spores, are all clearly demonstrable within a span of four days.

I have attempted to summarize the remarkable developmental pattern of the cellular slime molds and present as well methods of



FIGURE 5. Here we see a perfect "wheel" shaped pattern that is characteristic of the cellular slime mold aggregation pattern. This is viewed in a very "rich" culture of the mold.

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serv and and fifth isolation and maintenance. All of these experiences are within the grasp of any moderately equipped laboratory.

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Most important has been my effort to introduce you to a potentially important and perhaps unknown addition which might find a use in your laboratory. With these credentials offered on behalf of the cellular slime molds I predict a delightful acquaintance for you in the offing.

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Summer Graduate Courses in Botany

The University of North Carolina is now offering an expanded program of graduate courses in botany during its summer session for high school biology teachers under the auspices of the NSF Summer Fellowship Program. These are standard graduate courses that apply toward the M.A. degree. It should be possible to complete the requirements for an M.A. in a minimum of three summers provided some of the thesis is done during the year.

For further information contact Dr. Victor A. Greulach, Chairman of the Department of Botany, University of North Carolina, Chapel Hill. Application forms for NSF summer fellowships may be obtained by writing the Fellowships Section, National Science Foundation, Washington 25, D. C. Completed applications must be in to NSF by January 5, 1962.

AIBS Visiting Biologists Program

More than 135 scientists in 34 states will serve this year as Visiting Biologist lecturers and consultants in colleges, small universities, and high schools. The AIBS, announcing the fifth year of the program for colleges, said applications for 1961-62 participation are now being received.

The program, supported by grants from the National Science Foundation and the Atomic Energy Commission, provides for visits by prominent biologists for participation in seminars, lectures, consultation with faculty, and scientific or career discussions with students. Visits normally are for three days.

A similarly supported program for high schools will be operated for the third year.

A third program, by which an institution may invite a particular specialist from abroad to visit, will be operated for the second year. This plan allows foreign biologists to visit at least three colleges, laboratories, or other scientific centers over a period of a month.

Information on all programs can be obtained from Miss Martha J. Acker, American Institute of Biological Sciences, 2000 P St., N. W., Washington 6, D. C.

Geographic School Bulletin

The National Geographic Society announces a new and improved *School Bulletin* for elementary schools. It may be obtained from the School Service Division, National Geographic Society, Washington 6, D. C., for \$2.00 for 30 issues.

Effects of Radiation on Germination Patterns of Spores of Certain Mosses and Ferns

· C. Richard Snyder, Radnor Senior High School, Radnor, Pennsylvania

Introduction

This study was carried on under a grant from the Heart Association of Southeastern Pennsylvania during the 1960-61 school term in the laboratories of Radnor Senior High School.

The work was all done after school, and the students involved were Samuel W. Warburton, Jr., Kelsey Brown, and John Claggett, Seniors; and Carol Morse, Robin Thomason, Carl Asher, and Peter Shelley, Juniors.

Grateful acknowledgement is made to these students, as well as to various scientists and engineers who gave valuable advice and assistance to the project, and without whose help the completion of the work would not have been possible. Dr. Seymour Shapiro, of the Biology Department of Brookhaven National Laboratories, Upton, Long Island, New York, was responsible for all of the irradiation of the spores. Dr. John M. Fogg, Jr., Director of the Morris Arboretum of the University of Pennsylvania, gave valuable advice and aid. Mr. Robert O. Nover, of Burroughs Corporation, Radnor, Pennsylvania rendered invaluable technical assistance, and Mr. Stephen Colalongo, bacteriologist with Wyeth Laboratories, of Radnor, Pennsylvania, contributed help with working out some of the problems connected with the culture medium used to germinate the spores.

Preliminary Investigations

Certain studies had to be made, prior to the irradiation and germination of the test spores, in order to determine (1) what species of ferns and mosses were most suitable to use, and where they could be obtained, (2) what culture medium would produce the best growth, while discouraging the growth of contaminants, particularly molds, (3) what were the optimum environmental conditions for the rapid and complete germination of the spores.

The original plan was to use spores from

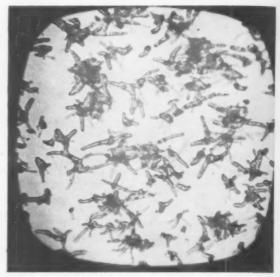
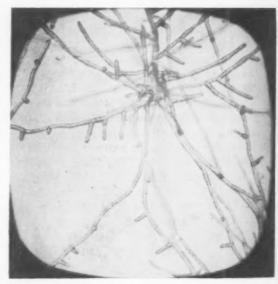


FIGURE 1. 100X-1/2 sec.



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FIGURE 2. 430X-1/2 sec.

plants growing in local wooded areas, but this was abandoned because of the large number of colonies of molds and bacteria that appeared on the plates soon after seeding. The initial plan also called for the use of

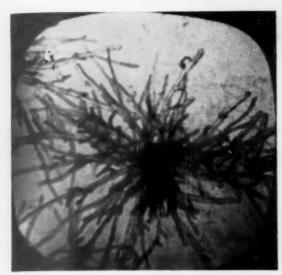


FIGURE 3. 30X-1 sec.



FIGURE 4. 100X-1/2 sec.

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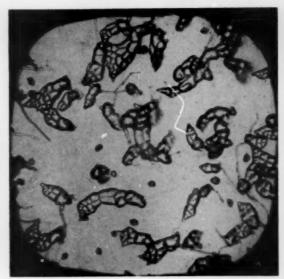
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triptocase glucose extract agar, but this was replaced by a commercial preparation especially designed for the germination of moss and fern spores. The ferns and mosses were secured from a commercial supplier. The moss used was Polytrichum commune, and the fern was Woodwardia virginica. The spores were germinated in standard petri dishes, near a sunny window, directly beside a heater-ventilator unit.

Technical Information

The isotope used was phosphorus-32. Ten



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FIGURE 5. 100X-1 sec.

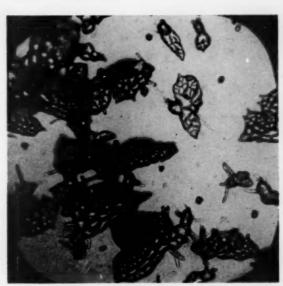


FIGURE 6. 100X-1 sec.

microcuries, in the form of H₃PO₄, in one ml. of N/100 HCl, was the original quantity. This was diluted 1:10, and was used approximately 2 half-lives from original activation.

The spores were irradiated at Brookhaven National Laboratory by X-rays, in varying dosages, as will be noted later.

The photographs were made with a Robot Vollautomat Star II camera, through a Beck-Kassel microscope, equipped with a Sixti light meter. Adox KB 17 film, ASA rating of 60 was used. Exposure time varied with the thickness of the material, and the magnifica-

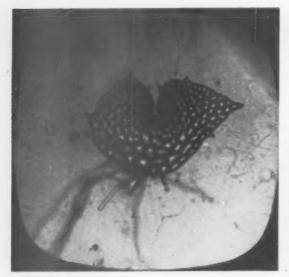


FIGURE 7. 30X-2 sec.

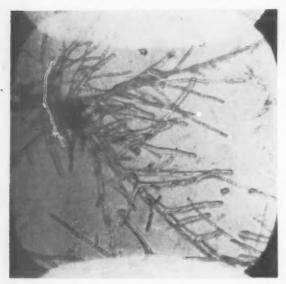


FIGURE 8. 430X-1 sec.

tion varied with the amount of the material that could be shown in the field. Exposure time and magnification are indicated with each photograph.

Procedure and Methods

Many investigators have studied the effects of ionizing radiation on plants. A bibliography (1) published in 1958 by the Brookhaven National Laboratory has almost 2600 entries dealing with work done in this field between 1896 and 1955. Very little work has been done on mosses and ferns and practically all of it in Germany and Russia. The most recent

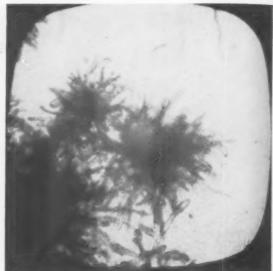


FIGURE 9. 100X-1 sec.



FIGURE 10. 100X-1 sec.

entry is dated 1952 and states that Breslavets investigated the effects of X-rays on fern prothallia.

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The purpose of this present project was to study the effects of X-radiation of varying dosages on the germination pattern of the spores of mosses and ferns from the time of the first bursting of the spore coat to the beginning of the gametophyte. A secondary purpose was to investigate the changes in the germination pattern brought about by the presence of isotopes, either on the surface of the medium or dissolved in it.

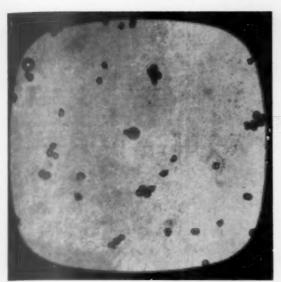
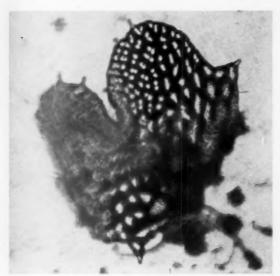


FIGURE 11. 100X-1/2 sec.



Fugure 12. 100X-2 sec.

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After the spores had been irradiated, the dishes were seeded, as follows: control (no radiation), 100, 200, 300, 400, 500, 1000, 2000, 3000, 4000, and 5000 roentgens, with and without isotope, one dish of moss spores and one of ferns. Duplicates were seeded for all dosages, making a total of 88 plates that were observed daily. Photographs were taken at intervals when significant changes were noticeable. In all, more than 300 photographs were made. Twenty of them have been included in this report.



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FIGURE 13. 30X-2 sec.

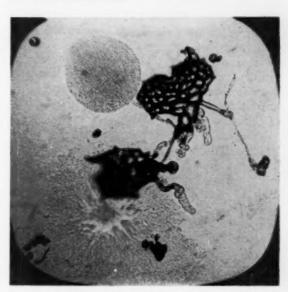


FIGURE 14. 30X-1 sec.

Results

Campbell (2) has described the normal development pattern of the moss and fern spores. The control spores of this experiment illustrate this pattern well, as can be seen in Figures 1 to 8.

In mosses, the spore absorbs water and begins to enlarge until the exospore is burst when the endospore protrudes as a papilla which grows into a filament. If abundant moisture is present, the protonema grows with great rapidity and may form a dense, branch-

ing, alga-like growth of considerable extent. These developments are seen in Figures 1, 2, and 3. Figure 1 shows the extent of development at the end of three weeks growth, Figure 2 shows the growth at the end of four weeks, and Figure 3 demonstrates the dense, alga-like growth found at the end of nine weeks development. The development of the fern spore is somewhat different. The rupture of the exospore allows the papilla to protrude, as in the moss, but the prothallium is formed by the longitudinal division of the filament which eventually produces a spatulate form, and it becomes heart-shaped by the rapid growth of the outer cells of the young segments. These developments are shown in Figures 4-8.

Figure 4 shows the development of the filament by the end of two weeks. In Figure 5 the fern has grown an additional week, and the spatulate form of the developing prothallium is evident. Figure 6 (four weeks growth) shows all stages of the fern's germination from undeveloped spores to almost-mature prothallia indicating that there is by no means a uniform germination. Figure 7 indicates a 9-week-old prothallium, which is clearly heart-shaped, and which shows the beginnings

of rhizoids.

Time of initial germination. In the non-irradiated controls, the fern spores began to germinate in 4-5 days, while the moss spores' initial germination was not evident for 9 days. Ionizing radiation delayed the growth beginning by an average of 2 days in the fern and an average of 4 days in the moss. There appeared to be no correlation between the period of delay and the amount of radiation.

Percentage of spores germinating. No noticeable effect was evident on any spores given less than 300 r dose. From this point, however, through the 4000 r-irradiated spores, the percentage of the spores that had been sown on the plates that germinated dropped as the dosage increased, and at 5000 r germina-

tion ceased completely.

Visible effects of radiation. In the moss, a 400 r dose produced little effect upon the normal growth of the protonema (Figure 8). However, a 1000 r dose produced much clumping of the spores and a drastically reduced lengthening of the prothallium (Figure 9).

This effect was even more pronounced in the moss spores which had been given a 4000-r

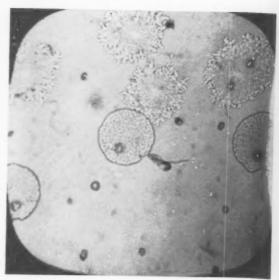


FIGURE 15. 100X-1/2 sec.

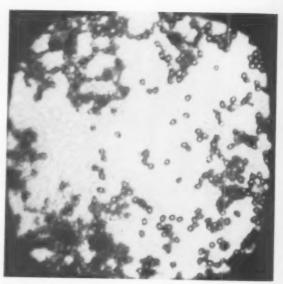


FIGURE 16. 100X-1/2 sec.

dose (Figure 10). As mentioned above, 5000 r seems to be a lethal dose for the moss spores. No noticeable growth is evident in Figure 11. All of these pictures were made 4 weeks after the initial sowing.

Ferns whose spores had been given 400 r (Figure 12) and 500 r (Figure 13) grew vigorously and showed complete prothallia in

4 weeks time.

In Figure 13 it may be noticed that there are many ungerminated fern spores in the field. This shows the effect noted previously under "Percentage of spores germinating."

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FIGURE 17. 30X-1 sec.

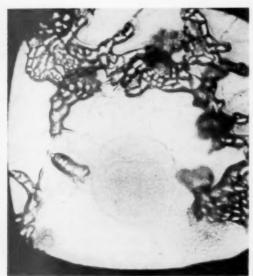


FIGURE 18. 30X-1 sec.

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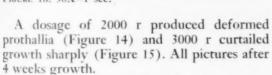
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Again, 5000 r proved to be too great a dosage for the spores to survive (Figure 15). It must be stressed that no irradiation was administered after germination had begun. All X-raying was done on the spores alone. A vigorous growth of deformed prothallia took place in what was recorded as a plate of 4000 r spores. There is no explanation for this.

The isotopes were administered in two ways: (1) Dropped on to the surface of the

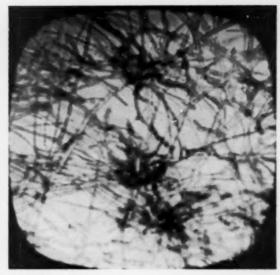


FIGURE 19. 100X-1/2 sec.

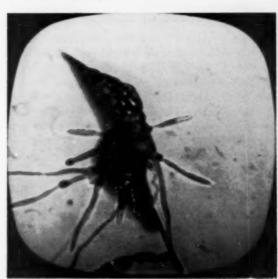


FIGURE 20. 30X-2 sec.

agar, where they caused a discoloration of the agar, appearing in the pictures as spots and (2) dissolved in the agar. The effects of the spots of isotopes are visible in Figure 17 (moss) and Figure 18 (fern). In both cases, the growing spores are seen to avoid the isotope. Figure 17 shows growth after 9 weeks, and Figure 18 growth after 4 weeks.

The effects upon the growing plants after 6 weeks of germinating in agar in which the isotope had been dissolved are shown in Figures 19 (moss) and 20 (fern). The moss protonema is noticeably thinner than would be expected, and the fern prothallium is de-

formed. Neither group of spores had been X-rayed. The activity of the isotope was so low that it could not be recorded as any significant number of counts per minute above background on our Geiger counter.

Figures 19 and 20 show the effects of dissolved isotopes on the growth of the protonema of the moss (19) and the prothallium of the fern (20).

Conclusions

1. Ionizing radiation (a) lengthens the time required for germination to begin, (b) reduces the percentage of spores that germinate, (c) causes abnormal growth of the germinating protonema or prothallia as long as 9

weeks after the exposed spores have been sown.

2. Dissolved isotopes (a) are avoided by germinating spores (b) cause, when avoidance is impossible, deformity and abnormality in growth patterns.

3. These conclusions hold true for both moss and fern spores.

Bibliography

- Sparrow, Arnold H., Binnington, John P., and Pond, Virginia. Bibliography on the Effects of Ionizing Radiation on Plants. July, 1958. Brookhaven National Laboratories, Upton, New York.
- 2. Campbell, J. H. Mosses and Ferns. New York, Macmillan Company, 1918.

"Vistas of Science"

A new series of paperback books to introduce high school students to key areas of science will be issued beginning this fall by NSTA and Scholastic Book Services, a division of Scholastic Magazines, Inc. Each book in the series will consist of three sections: a summary of what scientific research has discovered about a field of science, unsolved problems, and projects on which students can work. The 128-page books will be abundantly illustrated with diagrams, charts, and photographs.

Three books are expected to be completed by the first of the year: The Living Cell (tentative title), by William Deering, associate editor of Science World magazine; Spacecraft, by James J. Haggerty, Jr., Aerospace Writers Association; and Measurement: The Basis of All Science (tentative title), by William J. Youden, Sr., National Bureau of Standards. Other books are planned in such areas as dental research, ceramics, biochemistry, molecular biology, space biology, astronomy and cosmology, water and metallurgy.

Post-Doctoral Fellowships

Five fellowships are open to those entering or intending to enter careers in college or university administration. To qualify for a fellowship the applicant must already possess his doctor's degree (the field of academic study is immaterial), or the equivalent, and already have demonstrated leadership potential. Fellowships are graduated according to need but with a maximum of \$8,000. The applicant should be no more than forty years of age and able to provide a good bill of health.

For application forms and further information address: Center for the Study of Higher Education, The University of Michigan, Ann Arbor.

Local Science Meeting

A science-mathematics conference for public school teachers and administrators to review and discuss study programs being developed by national committees was held Saturday, September 30, 1961, at Luther College, Decorah, Iowa. Dr. Bentley Glass, The Johns Hopkins University, and Chairman of the Biological Sciences Curriculum Study, spoke. Section meetings were conducted by representatives of the study programs.

Women in Biology Leaflet

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As another service of NABT, copies of a new leaflet produced by the U. S. Department of Labor, Women's Bureau, has been mailed to all NABT members. It is hoped that this will be of great benefit to those teachers wishing to counsel girls in the possibilities for careers in biology.

Root Tip Cell Squashes for the Study of Cell and Chromosome Morphology

. William H. Miller, Maine West High School, Des Plaines, Illinois

Root tip squashes from actively growing root tips provide fine material for the study of cell and chromosome morphology. High school biology students can be shown this technique in a short time and are able to distinguish many cell structures which are sometimes overlooked in routine laboratory work. The chromosomes are fundamental biological structures which should be seen first hand by every biology student.

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The following technique for root tip squashes is in general a modification of that used by Tjio and Puck (3) and has been used with good results by the author on chromosome studies of *Matthiola incana* (2) and subsequently used as routine biology laboratory work by high school biology students.

Obtaining material for study

It is very important to have healthy, growing root tips. The best tips can be obtained by repotting plants for study in a slightly larger pot and after 4-6 days collect the new tips emerging from the new pot soil. Good tips may also be obtained by germinating seeds or rooting cuttings. It is also possible to dig or pull plants directly from soft or wet soil and after washing, obtain good tips. In this way species observed on field trips can provide material for a good follow-up laboratory.

Pretreatment

It is advisable to place the tips directly in .1% aqueous solution of colchicine and refrigerate at 3-5° C for 5-7 hours before squashing. A saturated aqueous solution of 8-hydroxyquinoline may also be used in place of the colchicine. These solutions influence the spindle fibers of cells in metaphase and arrest cell division at this stage. By use of this pretreatment many metaphase cells can be obtained on one slide and greatly facilitates the students' work. The colchicine treatment may shorten the chromatids and cause them to part somewhat, but this is good, for the shortening results in a more heavily stained chromosome.

Fixation

After this pretreatment the tips should be fixed in 3 parts alcohol: 1 part 28% acetic acid overnight before squashing.

Prior to squashing, the tip should be brushed lightly with a fine brush dipped in fixative. This removes any soil particles which would interfere with squashing.

Stain-hydrolysis

A 2% acetic orcein stain is prepared as outlined by Tjio and Puck (3), by dissolving 2 gr of synthetic orcein in 45 cc of boiling glacial acetic acid with stirring. The solution is allowed to cool to 50° C and 55 cc of distilled water is added slowly. After cooling the solution should be filtered twice and occasionally thereafter while in use. Root tips must be hydrolyzed prior to squashing. This will soften the tissues by dissolving the intercellular cement. The hydrolyzing solution is prepared by mixing the following in this order.

- 3 parts of the previously made 2% acetic orcein stain
- 1 part of previously made fixative
- 1 part water
- 1 part con. HCl

Place 2-5 good tips in a solution of 5 drops of 2% acetic orcein and 1 drop of hydrolyzing solution in a watch glass. Heat the solution over a spirit lamp until vapors appear at the surface, set it aside for 2-5 minutes to obtain the desired degree of softening. If additional softening is needed for large tips more hydrolyzing solution may be added or the time may be increased. Transfer the tips to a second watch glass which contains several drops of 2% acetic orcein and heat over the spirit lamp until vapors appear at the surface, set aside for 10 minutes. This additional time in 2% stain greatly improves the staining.



514

FIGURE 1. The 14 chromosomes of virescent double *Matthiola incana* 1800X.



FIGURE 2. The 18 chromosomes of prickly lettuce Latuca virosa 1200X.



FIGURE 3. The 36 chromosomes of common ragweed Ambrosia elation 1200X.



FIGURE 4. The 36 chromosomes of cocklebur Xanthium italicum 1200X.

Making the squash

Remove the heavily stained portion of the root tips and place them on a clean slide in a small drop of 2% acetic orcein. Using a needle or flat spear point, crush and tear apart the root tips. If the tips are sufficiently hydrolyzed, they should literally fall to pieces under the needle.

Cover with a clean, lint free, cover slip, Place the slide, cover slip down, on a filter paper and with light thumb pressure blot away excess stain. Squashing may be accomplished by light tapping of the cover slip with the smooth handle of a dissecting needle. It is useful to press the handle to the slip and circle it around several times over the tissues to squash and spread them. With the cover slip up, and filter paper on both sides of the slide, use moderate-strong thumb pressure to increase squashing. Replace any stain necessary and make preliminary examination to see if the cells are squashed adequately. If so, seal the cover slip with Krönigs cement or similar mastic. If the cells are not squashed to sufficiently disperse the chromosomes within the cell, use mild heat with the spirit lamp and additional thumb pressure. If this does not help, prepare another slide.

The stain should clearly differentiate the nuclei of resting cells and heavily stain chromosomes of dividing cells. The nucleolus is also usually visible. Students with good spreads of chromosomes may proceed to count them and possibly pick out distinctive homologous pairs. If equipment is available the good spreads may be photographed and drawn.

The slides when sealed and refrigerated will keep in good condition for several months; in fact the staining will improve after a few days of refrigeration. The slides can be made permanent by use of the McClintock (1) method or according to Tjio and Puck (3).



FIGURE 5. Photomicrograph of the somatic chromosomes from the root tip of the eversporting, green single Matthiola incana. It has been shown (unpublished) that this form has a heteromorphic pair of homologous chromosomes due to a deficiency on the short arm of one of the indicated pair.

These procedures are rather time consuming and tedious for general laboratory work and would be recommended only as an additional side project for students with the time and interest. If the slides have not been sealed, or if the mastic can be removed, acceptable permanence can be quickly carried out by simply placing a drop or two of venetian turpentine near the edge of the cover slip and setting it aside for several weeks.

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noeen unpair ncy This technique will open a new avenue of study for many students, and side projects will quickly be suggested after the technique is learned.

Literature Cited

 McClintock, B. A method for making acetocarmine smears permanent. Stain Tech. 4: 53-56, 1929.

- Ross, J. G. and W. H. Miller. Identification of somatic chromosomes in eversporting type of *Matthiola incana*. Submitted to Jour. of Heredity.
- Tjio, J. H. and T. T. Puck. Genetics of somatic mammalian cells. II Chromosomal constitution of cells in tissue culture. Jour. Exp. Medicine, 108: 259-268. 1958.

Suggested Reading and Reference

Chromosome Atlas of Cultivated Plants, C. D. Darlington and E. K. Janaki Ammal, 1945, George Allen and Unwin, Ltd., London.

The Handling of Chromosomes, C. D. Darlington and L. F. LaCour, 3rd. ed. 1960, Macmillan Co., New York.

NABT Teacher Award Program

President Paul Webster has announced details of a Teacher Award Program, sponsored by NABT, for the selection of An Outstanding High School Biology Teacher in each state. A Regional Award will be given the nominee from one of the states in each Region, and this person will be given some recognition by the AIBS. A committee consisting of President Webster, Region IV Director John Gundlach, Philip Fordyce, and Editor Paul Klinge, formulated the plans for the awards.

Each Regional Director will be responsible for the appointment of a State Director for each state in his Region, and this person will organize a committee for the promotion and selection processes of the program within that state. When state winners are selected, the Regional Director will chair a committee of all the State Directors to determine the Regional winner from among the State winners.

The State Director and his committee will be the key persons in this program. They are responsible for publicity of the program, distribution of application forms, and selection processes. Their names will be announced shortly. The application form will consist of a nomination blank which can be completed by school administrators, department heads, supervisors and consultants, college or industrial biologists, other teachers, or the candidate himself; a personal data blank for the nominee; and the recommendations from persons in the education profession. After the State Committee has reviewed the completed forms, they will select from 3 to 5 nominees and visit them in their teaching situations. They will then determine the State Award Winner from these.

The time schedule for the program is as follows: Blanks ready for distribution, January 1; completed blanks due to State Committees, March 1; State winners announced; Regional winners announced, May 1.

Watch for announcements of your state organization plans.

Experimenting With Planaria

• John Gabriel Navarra and Donald R. Cicero Jersey City State College, Jersey City, New Jersey

Biology as it relates to general education at the high school level and the college level should concern itself with and develop in students the spirit of inquiry and intellectual groping. The suggestion for experimentation in this article develops an approach for using ordinary laboratory animals to experiment with conditioning, learning, and the relationship of animal behavior to the complexities of the central nervous system.

The fresh-water planarian, *Dugesia tigrina*, is the animal selected for this experiment. The planarians might be obtained from a pond or stream. Of course, a biological supply house provides a convenient source. Part of the experimentation can very well include the search for and isolation of the animal. The materials used in the experimentation are easily obtainable: a six-volt battery, flashlight bulbs, knife switch, bell wire, push-button type electrical switch, aluminum foil, spring water, and beakers.

Experimental Procedure

About thirty planarians should be placed in a beaker filled with spring water. Two electrodes made with aluminum foil are arranged on either side of the planarians so that it is possible to shock them by using the current from a six-volt battery. A push-button is used to make and break the circuit. Simultaneous with each electric shock, a flashlight bulb, placed over the jar, blinked on and off. This treatment was repeated for three hundred consecutive times within an interval of one hour. It will be noted that on each occasion of shock, the planarians respond by a gross body contraction.

A control group of five planarians did not exhibit similar gross body responses to the flashing of the light alone. The absence of the occasion of the electric shock appeared to leave them unconditioned.

The "unconditioned" and the "conditioned" planarians are referred to as Group 1 and Group 2 respectively. These planarians can then be involved in an extension of the experimental design. Fifteen planarians in the conditioned group were cut in cross section close to the anterior end. The other portion of the conditioned group were cut in cross section close to the posterior end. We now have head

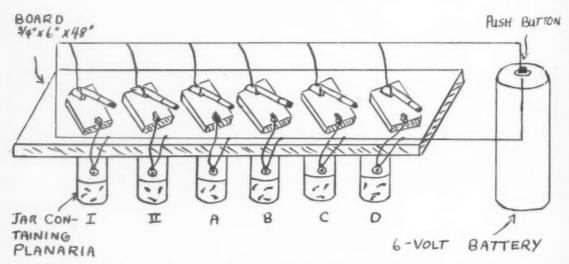


FIGURE 1. Once individual knife switches were closed, the various groups of planaria could be subjected to the flashing of light as controlled by the push button. In this manner, it became possible to determine which group of planaria had retained the original conditioning.

and tail sections for each individual planarian. Each section will regenerate the missing part, i.e., the head section will regenerate a tail and the tail will regenerate a head.

Separate the dissected planarians into groups:

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- a. Group A consists of the head section of this group. The head section would be the smaller part as a result of the dissection.
- b. Group B consists of the tail section of this group. The tail section would be the larger part as a result of the dissection.
- 2. Planarian dissected close to posterior end.
 - a. Group C consists of the head section of this group. The head section would be the larger part as a result of the dissection.
 - b. Group D consists of the tail section of this group. The tail section would be the smaller part as a result of the dissection.

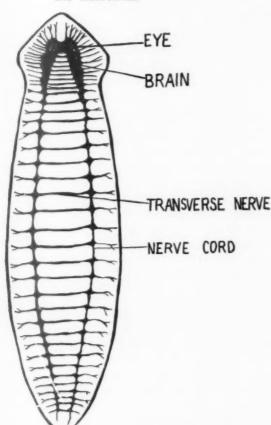


FIGURE 2

The sections of the planarians placed in Groups A, B, C, and D each regenerate the portion of the body which was amputated as a result of the dissection. It should be remembered that each of the planarians in groups A, B, C, and D have an original portion which was conditioned. For example, the head section of Group A belonged to a "conditioned" planarian.

The planarians in groups A, B, C, and D were tested to determine if they retained the conditioning. The light was flashed over each jar of planarians. The conditioning as you will remember is the gross body contraction when the light flashes.

Planaria Retested Strength of Reaction

Group 1	No Reaction
Group 2	Similar to initial trials (fully conditioned)
Group A	Slight contraction noted in a few sporadic cases
Group B	Slight contraction noted in numerous cases
Group C	Gross body contraction (fully conditioned)
Group D	No contraction noted

Results of Experiment

The experiment indicates that planarians can be "conditioned." In addition, there is every indication that the planarian will retain the conditioning, even after regeneration has taken place. However, the extent of retention of conditioning depends on a number of factors.

Student Experimentation

Experimentation of this type can open up many avenues of inquiry and speculation for the student. Especially as they search for a framework of hypothesis and theory to begin to make the data intelligible.

Student inquiry usually starts with an examination of the nervous system. The nervous system of the planarian consists of two cerebral ganglia. This "brain" is connected with two lateral, longitudinal nerve cords extending posteriorly. The nerve cords are connected with each other by transverse nerves.

Metabolic activity is another significant point toward which student investigation is directed in the inquiry related to the experiment. Many new techniques must be developed by the student to test out his "hunches" and hypotheses. And new data accumulate!

Implications of the Experimentation

Biology as it relates to general education at the high school level and college level should concern itself with the method of inquiry, and this can be done effectively through a consideration of the relationships of life and structure in life to the total environment.

Many primitive animals in both the invertebrate and vertebrate phyla have remarkable powers of regeneration and can be employed in similar experiments. A few ex-

amples are earthworms, crayfish, starfish, salamanders, and lizards. It is, of course, known that the power to regenerate is greatest in young animals. For this reason, comparisons between animal age and retention of conditioning might also prove to be an interesting and rewarding avenue of research.

Such investigations may lead to additional clues regarding the nature of neural transmission and inherited behavior patterns. Certainly, there are implications for man which are both dramatic and far-reaching. While it is obviously true that humans cannot regenerate like planaria or starfish, neural conditioning of this sort plays an important role in intellectual development. There have been indications that research of this type suggests a chemical basis for the transmission and retention of nerve impulses.

Have You Used Phluoroglucin Solution?

· Wayne E. Manning, Bucknell University, Lewisburg, Pennsylvania

I consider phluoroglucin solution (phluoroglucinol) one of my most valuable chemicals, and it is never absent on my reagent shelf.

This is only a test for one thing, lignin, but for this purpose it is extremely valuable. It is used in my teaching of botany courses in demonstrating regions in the cross sections of the stem and in demonstrating wood products

such as paper.

A study of the cross section of the woody stem, or of the mature herbaceous stem, may of course be demonstrated by blackboard drawings, charts, kodachrome slides, or studies of slides under the microscope. However, a student often fails to relate any of these to the actual stem itself and often has a complete lack of knowledge of the comparative size of pith, wood, and bark, especially the latter. If one cuts up into short pieces a twig, one year old or older as preferred, (basswood, elm, etc.) and gives each student a piece, and then puts on each section of wood (not clothes) a drop of phluoroglucin solution, the xylem will color a definite red and will contrast sharply with pith and bark. It should be re-

membered that phloem of many woody plants contains phloem fibers, and there is often a cap of phloem fibers (formerly called pericycle fibers) over the phloem in such herbaceous stems as sunflower (Helianthus). Since these fibers have lignified cell walls these will usually stain red as do the xylem vessels and xylem fibers, but the relative amount of phloem fibers is so much less than the xylem that there is usually very little confusion in demonstrating the regions. Furthermore, there is a decided advantage in indicating the presence and location of lignified tissues in the phloem. Thin hand-made sections of a stem on a microscope slide can be treated with the solution and studied under the microscope. The presence of lignin in any piece of wood may, of course, be easily demonstrated with the chemical.

It is worthwhile noting that the color of lignified tissues in most botanical slides is also red, the result of the dye saffranin. Thus there is a definite correlation between study of fresh stems and prepared slides or even of projected kodachrome slides. However,

saffranin is not satisfactory as a test as used above and below.

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The economic uses of plant parts should always be taught in a course in botany or biology. Paper is one of the most important of these uses. All ordinary paper is made from wood, and in general from spruce wood, or pine (or at least from conifers, softwoods), though some of our specialized papers (book papers) are made from Angiosperm wood (hardwoods). Newspaper and cheaper paper toweling are made, at least in large part, from ground up conifer wood with very little following chemical treatment. Writing paper and most other grades of paper are made from wood cells separated from each other by dissolving of the connecting primary walls by some chemical, such as sulphurous acid. In this process of digestion of wood the lignin is dissolved. A very simple test with a few drops of phluoroglucinol on newspaper, and on writing paper, will very quickly demonstrate the presence of lignin in the former and the absence of lignin in the latter. Even if one does not take the trouble to demonstrate the actual making of a sheet of paper in class (this is done in one or two hours in my classes), the nature of paper is important to teach.

If one wishes to continue interesting studies of the making of paper, one should drop iodine on the two kinds of paper. Writing paper, and especially drawing paper, has a surface sizing treatment with starch for the actual purpose of writing, and the presence of this quickly shows up. Of course, newspaper is not used for writing, and starch is usually absent.

There are a few notes necessary. Phluoro-glucin is a test, not a stain; consequently, permanent slides cannot be made from sections of stem treated with the solution. The solvent is alcohol and hydrochloric acid, so water does not mix at all well with this on a microscope slide, and the solution may evaporate rather quickly. The solution should not touch the parts of a microscope nor the clothes. It is, of course, well known that younger parts of stems may not be mature, and lignin may not yet have developed in these parts. Some kinds of stem are so moist that the solution somehow does not appear to show

up the lignin very well, so it is necessary to try out the material in advance.

Phluorglucin solution (phluoroglucinol) was formerly considered completely unstable, and was supposed to be made into a solution each time one used it by dissolving the material in alcohol, etc. This was a nuisance. The material is now available as a solution from some supply houses (A. H. Thomas and others) for about one dollar. This solution will keep for a year or so, though it will gradually turn brown and become less effective. The bottle, of course, should be kept tightly corked.

Try it once. It is possible that phluoroglucin may also prove valuable in use in cross sections of leaves, or in use with commercial fibers. I have not tried these to any extent.

NSF Graduate Fellowships

Applications for these fellowships are due January 5, 1962, for advanced study in the sciences. Application material is available from the Fellowship Office, National Academy of Sciences, 2101 Constitution Avenue, N. W., Washington 25, D. C.

John Hays Fellows

The John Hays Fellows Program is receiving applications for academic year fellowships for secondary school teachers. Applications are due December 1, 1961. Further information may be obtained by writing to the program office, 9 Rockefeller Plaza, New York 20.

AIBS Grant

The Biological Sciences Communications Project, a new activity of the AIBS, has been awarded a \$151,200 grant by the NSF. Dr. Charles W. Schilling, Project Director, states that the mission of the new project is to study carefully all steps in the flow of information from the person who produces it to the person who uses it.

Experimental Approach to the Study of Fresh Water Organisms*

ERNEST LITWEILER, John Adams High School, South Bend, Indiana

A teacher of biology may follow a plan of outlined procedure, meet all the state and local curriculum requirements, and still teach a dead subject. Biology is a living science, and we believe that it offers an opportunity for instructors to instill creativeness, a high regard for the orderliness of nature, and an enthusiasm for science at the high school level.

Students are drawn to the study of aquatic biology by the urge to seek the unknown, by the fascination *Homo sapiens* has for water, by the grotesqueness of some organisms, or by the sheer beauty of microscopic plants.

We believe that it is not unusual that youngsters should enjoy the study of fresh water biology. The collecting, observing, classifying, and drawing activities are closely akin to hunting, gambling, naming, and illustrating processes of primitive man. Students who elect to study biology have a right to expect to study living organisms and to view some of these phenomena in the microscope. They have a yen to experiment, handle, and observe organisms as they capture them.

The chances are that each spring there are some temporary ponds somewhere near your school. They may cover an area as large as your football field or they may be the size of a tea cup. It may be clear and sparkling, or it may be filled with debris. Its appearance is not as important as you might think. It is your gold mine for studying fresh water organisms.

Let's start out cold. Without previous preparation in what we are seeking, the class makes its first field trip carrying homemade nets and gallon jars. Samples are taken from the mud in the bottom of the pond, from the sub-surface, and surface water. Each student now has his own aquarium.

On that first trip the student may find the eggs of an amphibian, some adult or immature insect, a turtle, or a snake. Sometimes fairy shrimp, those ethereal upside down creatures,

are prevalent. These specimens are all taken back to the laboratory for further study.

The big surprise, however, comes when a sample of water is placed under the microscope. It may be teeming with microorganisms. Often green plant cells with flagella are seen swimming about with a motility as great as the protozoa, the crustacea, or the free living rotifers which make up the aquatic community.

At this time we should spend a class period or two reviewing plant and animal systematics and the niche that each occupies in the balance of water ecology.

After this review, the students are ready for the lab work for which they have been preparing. Only a few of the organisms are readily identified because the previous experience of these students in this line of endeavor has usually been limited. For this classification work we would highly recommend a book authored by Eddy and Hodson, Taxonomic Keys to Common Animals of the North Central States. The invertebrate keys in this book are useful for any section of our country. In this book are found hundreds of line drawings, which in our opinion, are most practical for the use of high school students beginning the intricate study of taxonomy of fresh water organisms.

The neophyte has a habit of jumping at conclusions. He might be reminded of what happened to the proverbial dog which jumped at a mule's conclusion with disastrous results. Classifying takes patience and painstaking observation. In some cases the organism is classified down to species; in others the youngsters do well to place the plant or animal in its proper family.

The organism is drawn as it is viewed under the microscope or the microprojector. We believe that there is value in being able to observe the specimen and then reproduce it on paper. The drawing is made from actual observation and not a drawing from a printed page or "a drawing of a drawing."

^{*}Presented to the NABT meetings with the AAAS in New York in December, 1960.



Figure 1. Some of the larger organisms are collected by the use of nets. However, most of the microorganisms are found in the mud and debris at the bottom of the pond.

The instructor makes himself available in observing the specimen under focus and discussing the classification in question with the student. Only when, in the opinion of the instructor, the student has classified his plant or animal as definitely as possible is he allowed to reproduce in his notebook his observations.

A minimum number of drawings is required. Some years when there is a great deal of "bloom," the requirements are higher than others when we have weeks of inclement weather.

Before the unit is culminated we study fresh water vertebrates. Northern Indiana is dotted



Figure 2. A dissection microscope is invaluable in observing organisms which may be seen dimly with the naked eye.

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Figure 3. A microprojector, without a horizontal reflector, is a splendid instrument for observing some of the larger organisms.

by numerous glacial lakes. Many of the common game fish such as northern pike, walleyes, and large and small mouth bass are found here. There are numerous pan fish: the blue gill, crappie, many varieties of sunfish, warmouth bass, rock bass, and perch. Some cold streams are stocked with rainbow, brown, and brook trout. These along with amphibians are studied. Sometimes glochidia, the parasitic larval form of clams, can be found embedded in the fish. Parasitic nemathelminths are also observed in the intestinal tracts of fish. Incidently, these may be placed in bowls in the laboratory and kept alive for weeks.

A special project is required for each class



Figure 4. Each student has a wide mouthed gallon jar which he uses for collecting and storing his organisms.



Figure 5. This axolotl lost his external gills and developed lungs because of the induction of a thyroid extract into the water in which he lived.

member. Since the material for study is so varied, a student may choose to do his research on any one of a hundred subjects. Some of the research problems which were particularly successful are:

- Comparison of organisms of permanent ponds and temporary ponds
- 2. The study of algae and its growth in artificial media
- 3. Artificial parthenogenesis of frog eggs
- Acceleration of the metamorphosis of an amphibian by use of thyroid extract.
- 5. Quantitative and qualitative test of coliform bacteria in rivers
- 6. Life cycle of fresh water clam
- 7. Survey of fish species of a river
- 8. Regeneration of planaria
- 9. Regeneration of the hydra
- 10. Survey of aquatic insects of a stream
- 11. The hydrogen ion concentration of a pond and the organisms found therein
- Comparison of pond fertility, types, and populations of organisms

It has been our experience that youngsters become very much absorbed in this study. They ask permission to work on their "beasties" during their free time. After all, the pursuit and capture of an organism are as innately a part of primitive living as eating and sleeping. There seems to be an inborn instinct in man which draws him to the unknown. Each time he looks into a microscope he hopes to find another organism unknown to him. Our prehistoric ancestors developed vocabularies by giving descriptive names to things which they had found. Developing an articulate vocabulary is meaningful in growth. Thus we enjoy defining our collection results by proper designation. The cliff dwellers and cave men drew illustrations of animals on rocks. People like to draw things that appeal to their fancies. Youngsters enjoy studying fresh water organisms, so this process is a "natural."

We then follow our ancient ancestors in enjoying hunting, gambling, naming, and drawing fresh water organisms.

If a biology teacher yearns to have his students absorbed in their work, enthralled about their discoveries, and eager to achieve in the laboratory, then the study of fresh water organisms may be the right solution.

Aerial Spraying

Mass aerial spraying, intended for pest control, may actually be destroying the balance of nature, thereby creating a stronger pest menace. Dr. Henry D. Russell of Dover, Massachusetts, a naturalist at the Boston University College of Liberal Arts, stated that mass aerial spraying often contributes to pest insect increase by destroying predator populations that would normally control the undesirable insects. He states, "Biological proof is increasing that this spraying has been found responsible for extensive loss of wildlife, including fish, birds, and mammals."

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Citing areas of research that may open up new vistas in pest control, Dr. Russell feels the use of insect hormones and x-rays to prevent fertile mating or the encouragement of natural enemies of certain insect pests offer possible means of control without seriously upsetting the balance of nature.

Polarization of Cells and Crystals

· Ted Stopyra, Bulkeley High School, Hartford, Connecticut

Now you see it, now you don't. This is exactly what happens when you use polaroid filters in a microscope. The fascinating effects brought out with polaroid light are unbelievable, and such an experience should never escape a student in a science class.

Materials and Preparations

- 1. Microscope
- 2. Polarizing filters-film
- 3. Glass slides
- 4. Cover slips
- 5. Pipette

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- 6. Vegetable peeler
- 7. Iodine solution
- 8. Potato
- 9. Onion
- 10. Elodea plant
- 11. Geranium plant (use leaf and stem)
- 12. Sugar crystals
- 13. Salt crystals
- 14. Salicylic acid
- 15. Pepsin

Procedure

1. Polarizing the microscope.—A two-inch sheet of polarizing filter-film may be obtained from any scientific supply house for \$1.20. By taking the eye-piece out of the microscope, either by lifting or unscrewing, you cut a piece of polarized filter with scissors to fit the diameter of the eye-piece.

If the eye-piece does not lift out of the microscope, just unscrew the top eye-piece and deposit the polarized film down the barrel.

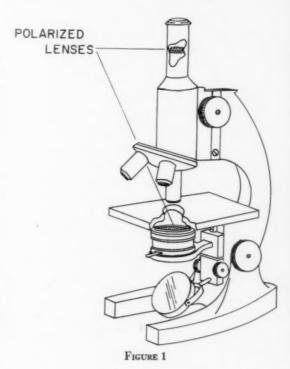
Once you have placed the polaroid film into the eye-piece, screw the top back on the barrel and proceed with the next step.

The other piece of polarized film is placed in the diaphragm disc or iris diaphragm. If the microscope has a diaphragm disc, cut a piece of polarized film to cover the largest opening on the diaphragm. Then apply the polarized film on the back of the diaphragm holding it in place with cellophane tape. For an iris diaphragm, place the cut polarized film on top of the diaphragm and the opening may be controlled to your own wishes.

When both polarized pieces are placed in their proper places as in Figure 1, the microscope is ready for use. The first step is to view and recognize polarized light. Set the microscope as if you were ready to view a specimen and then you rotate the eye-piece very slowly until a deep purple light appears. You now have polarized light. To return to white light, turn the eye-piece slightly either way.

Preparing the slides

a. Elodea leaf.—Place one leaf of an elodea plant on a glass slide with a drop of water and cover with a cover slip. Put the prepared slide under the microscope, focus, and observe under white light. Slowly turn the eyepiece to obtain polarized light, focus, and observe.



b. Onion skin.—Peel a small transparent tissue of an onion and place on a slide with a drop of water and cover with a glass cover slip. Focus the specimen under the microscope and observe under white light. Stain the onion skin with a drop of iodine solution and observe under white and polarized light.

c. Potato.—With the use of a vegetable peeler one may obtain a thin slice of potato. Deposit the potato slice on a glass slide with water and cover with a cover slip. Observe under the microscope first with white light

and then with polarized light. Stain the potato with iodine solution and observe the specimen again under both methods.

- d. Other fascinating slides.
 - 1. Cross section of geranium plant
 - 2. Stomates of geranium leaf
 - 3. Apple slice
 - 4. Salt crystals
 - 5. Sugar crystals
 - 6. Pepsin
 - 7. Salicylic acid

Dissection of the Common House Fly

• Darrell D. Young, State University of New York, Buffalo 22

The common house fly, *Musca domistica*, is too often ignored as a possible laboratory animal which may be used for dissection purposes. The fly is easily obtainable in large numbers at nearly any season, and specimens for laboratory work may be provided by the students themselves.

Although the common fly is often mentioned as a part of a biology course, its natural history and external morphology are too often forgotten. I have found that a more thorough understanding of these characteristics leads to a better understanding of the fly as a disease carrier.

The house fly belongs to the Class Insecta, Order Diptera. It is characterized by only one pair of wings, and one pair of balancers behind and below the wings. Without these balancers the insect cannot fly, since they act as a sort of gyroscope. The fly has two compound eyes, and is a true insect—having six legs. The fly has a keen sense of smell, and danger and food-getting seem to be inherited traits in it as well as all other insects.

The house fly will lay from 100-150 eggs at one time, up to 12 batches in a lifetime (one season), in refuse. These eggs will hatch in a day or two, depending upon the weather. The fly passes through complete metamorphosis, i.e., four stages; (1) egg, (2) larva, (3) pupa, (4) adult. The larva, or maggot as it is better known, lives in refuse dreading sunlight, there

feeding off of decaying material. It remains in this stage from 5-7 days before entering the pupa stage. The fly will remain in the pupa stage for approximately five days unless late in fall; then it remains in this stage throughout the winter thus providing for the continuation of the species. The adult house fly, upon emerging from the pupa stage, has expanders at the side of its head which aids the fly to reach the surface. It it fails to do so in a limited amount of time the expanders open, allowing the wings to spread, thus entrapping the fly within the refuse. One fly may be responsible for as many as one million descendents during the course of one year's time.

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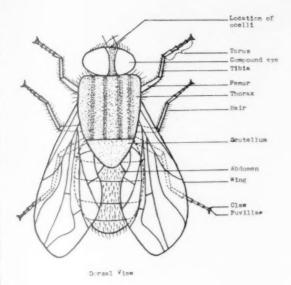
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The house fly has sucking mouth parts in the form of a proboscis (hollow tube-like tongue). Before eating solid food, the fly must convert it into a liquid which it accomplishes by regurgitating a fluid from its stomach which will dissolve the food. The fly then draws this liquid back up through the proboscis into its stomach. The familiar "fly speck" is left.

The fly can be of economic importance as a scavenger and in purifying the air by destroying carcasses and decaying vegetation. However, it has been known to carry and transmit many diseases, including typhoid fever, bacillary, amoebic dysentery, trachoma, cholera, and even tuberculosis. Also, it has been discovered that the house fly may pick



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up and harbor the poliomyelitis virus, although there is apparently no evidence at the present of their transmission of this virus.

I introduce the fly by having each student collect several specimens and bringing them to class. There are several reasons for this; (1) there will be no danger of a class member doing without in case he has forgotten to bring his specimens to class, (2) since the specimen is so small there is always a possibility that a part of it would have been smashed in the process of collecting the animal.

The day preceding dissection I spend one class hour in discussing its natural history and economic importance to man. Then, on the following day, the students will begin dissection. Each student will be required to dismember the animal and then with the aid of a microscope and a tripod magnifier, make the following drawings; (1) dorsal view, (2) wing, (3) foot, (4) proboscis, (5) cornea of eye. I have found that it is wise for the instructor to either purchase or make slides of the last four of the above. This will save the instructor endless time in explaining what the student is to look for.

The equipment needed for the dissection consists only of a scalpel or razor blade (single edge preferred) and a pair of forceps. The procedure to follow may consist simply of removing the various parts from the body of the animal by means of the forceps. The eye, however, may create something of a problem.

For best results, I have found that after the eye is removed it should be gently scraped on the inside to remove any tissues which prevent clear examination by means of the microscope.

It is also to be suggested that the laboratory study may be further extended by having the students make permanent slides, thus teaching them a laboratory technique beyond that of ordinary dissection.

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- The Encyclopedia Americana. Americana. Corporation, New York, 1954, pp. 352-354.

Book Reviews

General Biology

Cells and Societies, J. T. Bonner, 234 pp., \$4.50, Princeton University Press, Princeton, New Jersey, 1955.

This is not a new book, but the ideas which Prof. Bonner embodies in it as an elementary course in biology are new. Essentially, the author takes various types of animal societies and works backward until the cell level of organization is reached. Then there is a concluding section on metabolism and life functions. It is charmingly written, replete with examples and illustrations which should captivate the most captive audience. It seems to be light reading at first, but fundamental principles of biology are taught.

P. K.

GENERAL BIOLOGY, Revised Edition, Willis H. Johnson, Richard A. Laubengayer, and Louis E. DeLanney, 638 pp., \$7.95, Holt, Rinehart, and Winston, New York, 1961.

One of the college textbooks which high school teachers would do well to use as a reference in "beefing up" their courses. The drawings are original for this text, and they are superb. This is a straight-forward, no-nonsense book which comes to the point, and does it with detail.

The organization of the book includes introductory matter on the cell, followed by a fairly elaborate treatment of the anatomy of higher plants and higher animals. The chief life functions are taken up as they relate to the frog and man. The reproductive system is followed by the most elaborate treatment of embryology seen in a text. The last half of the book follows traditional systematics, beginning with the plants. Genetics, evolution, and ecology conclude the text.

The new material, not in the 1956 edition, includes a chapter on the ultrastructure of the cell and cell function. This includes some beautiful examples of electron microscopy and detailed charts of metabolic pathways. The material on photosynthesis has been revised and brought up to date. The genetics material is especially detailed, including population genetics, etc.

There is some material on bacteriology, improved over the 1956 edition, and with no emphasis on pathogenic types, but the other material is amazing in its inclusiveness. There are chapter-end questions and reading suggestions. The authors are to be congratulated on the book's straight-shooting and detail; students will not be at a loss to know what is available.

P. K.

Laboratory Manual for General Biology, Revised Edition, Willis H. Johnson, Richard A. Laubengayer, and Louis E. DeLanney, 181 pp., \$3.50, Holt, Rinehart and Winston, Inc., New York, 1961.

Written to accompany the text by the same authors, the manual covers a one-year course. The exercises are given in detail for student use, yet not too much detail. New exercises include some isotope experiments and some optional work. Drawings are furnished without labels. The chief vertebrate animal is the frog. There is a rather full survey of the plant and animal kingdoms.

P. K.

THE ENCYLOPEDIA OF THE BIOLOGICAL SCIENCES, Peter Gray, Ed., xxi + 1119 p., \$25.00, Reinhold Publishing Corp. New York, 1961.

The editor of this, the first true encyclopedia of biology in recent times, says in his introduction that the work is "intended to provide succinct and accurate information for biologists in those fields in which they are not themselves experts." He has achieved his aim. Specialists, reading articles dealing with their own fields

may feel that "their" subjects are treated superficially but at the same time find that the book serves their needs in other fields. The articles are written by experts from all over the world. These include such men as G. W. Beadle (biochemical genetics), F. A. Brown, Jr. (endogenous rhythms), T. Dobzhansky (genetics), P. P. Grassé (animal kingdom), J. Hutchinson (dicotyledons and monocotyledons), A. I. Oparin (origin of life), and S. A. Waksman (actinomycetes). To do justice to the book, this list should be extended to include all of the more than 400 experts who were contributors.

The articles range in length from about 500 words to over 5000. The illustrations are well chosen. Most of the articles have a list of references. There is a useful index. Interspersed through the book are a large number of brief,

unsigned biographical articles.

This is a book which should be on the reference shelves of libraries and biology teachers. The cost will discourage some, but it is suggested that a library with limited funds might well put a large part of its budget into a reference of this kind which covers the broad spectrum of biological subjects.

John M. Hamilton Park College Parkville, Missouri d

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Botany

FUNDAMENTALS OF PLANT PHYSIOLOGY, James F. Ferry and Henry S. Ward, 288 pp., \$6.50, The Macmillan Company, New York, 1959.

In their preface the authors point out that an elementary course in plant physiology is an appropriate addition to introductory work in botany. The availability of a suitable textbook for a one-term course in plant physiology has plagued many teachers, and such a book has been a long standing need. This book was written for students who have not had "organic chemistry, advanced physics, or higher mathematics." It might be added that the general level of presentation is so low that *no* college preparation in these areas would be required.

The authors have taken the traditional "shot gun" approach to study of an area of biology; they have attempted to touch on most topics in the area of plant physiology. Important topics are often treated with substantially less rigor than that in the high school books prepared by the BSCS. For example, the discussion dealing specifically with enzymes is given less than one-half page; buffers are not discussed, and pH is brought in only incidentally; though respiration is discussed (15 pages), no attempt is made to link cell ultrastructure to the process. No questions, problems, or suggested readings follow the chapters.

In the reviewer's experience, the topic of diffusion phenomena has always given elementary students difficulty. This frequently stems from a lack of understanding of random motion of molecules and the consequent "inevitable" motion or diffusion in most systems. Unfortunately, some common misconceptions, e.g., water molecules move only from high to low concentration, are not dispelled (though net diffusion is mentioned) and the authors state (p. 44): "Water will move from cell A to cell B [in the illustration] because cell B has the greater DPD, and not from the cell with the higher OP to the cell with the lower OP."

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Today there is increasing concern in biology with the nature of the living state. Plant materials can make unique contributions to the study and elucidation of problems involving cell metabolism, coordination of cell activities, and translation of the genetic code. It could not be said that this book will make evident to the student the important problems and ideas in the area of plant physiology. Let us hope that this is done by instructors using this book.

Joseph D. Novak
Department of Biological Sciences
Purdue University

Apical Meristents, F. A. L. Clowes, 217 pp., \$7.50, Charles G. Thomas, Publisher, Springfield, Illinois, 1961.

At a time when the genetic and "molecular" approaches to biology hold such a prominent place, it is comforting to know that there is still a substantial interest in the descriptive and experimental aspects of form and structure. It is, in fact, only in this area that the currently more glamorous studies find ultimate meaning. This little book brings together in concise compass and good perspective the investigations of numerous workers who, for half a century and more, have been concerned with what goes on in the developing tips of roots and stems. For the great majority of botanists the book will dispense with the necessity of consulting the numerous original papers widely scattered in time and space. In some places it clearly points to promising loci for future attacks on the problem, as, for example, in the discussion of the difficulties of trying to fit stem structure into the stelar theories. The book will probably be over the heads of most high school students, but it should find a place in every good school library. The indexes and the bibliography are excellent. We may wish that the halftone figures had been scattered through the book, to place them nearer the corresponding parts of the text, and that the different figures making up the plates had been separated a bit; but the typography, illustrations and manufacture of the book are otherwise excellent. This is Volume 2, but the first to appear, of a series of Botanical Monographs promised by the publisher.

Paul Weatherwax Department of Botany Indiana University

PHYTOPLANKTON OF CHESAPEAKE BAY, Ruth E. Griffith, 79 pp., Monograph No. 1, Hood College, Frederick, Maryland, 1961.

This spirally bound booklet will prove useful as an illustrated guide to the genera of algae comprising the phytoplankton of the Chesapeake Bay area. The majority of the genera belong to the blue green algae, the diatoms, and the dinoflagellates; only 8 genera of the green algae are recorded. This report is based on the records of many investigators whose names are listed following each species name in the account of each genus. Each genus is represented by one or more drawings which illustrate its basic features. A list of references to general taxonomic works is given. The key to the genera will be most helpful to the beginning student of phytoplankton.

R. C. Starr Department of Botany Indiana University

Zoology

Animal Diversity, Earl D. Hanson, 116 pp., Foundations of Modern Biology Series, Prentice-Hall, Inc., Englewood Cliffs, New Jersey, 1961.

This number in the famous series looks at the animal kingdom in a taxonomic way. But the title is a tip that the approach is far from orthodox. For one thing, the new systematics approach is used rather than the pigeon-holing technique so often found in most elementary texts. The author approaches the subject as a scientific problem and proceeds in a careful manner to examine the evidence for his initial hypotheses. First, the obvious animal diversity is shown; then, an explanation of the new systematics in relation to evolutionary thought. Temporal diversity is discussed as paleontology; spatial diversity as ecology; phylogenetic diversity is in greater detail. A carefully worded conclusion is a real gem. Highly recommended.

LIFE IN THE AQUARIUM, Philip Barker, 162 pp., \$3.50, Charles T. Branford Company, Newton Centre, Massachusetts, 1961.

The U.S. edition of this book, published in London by G. Bell and Sons, Ltd., will be of interest to aquarists, both general and specialized. The 23 chapters included discuss the establishment, stocking, and maintenance of temperate, fresh water tropical, and marine aquaria. Information included, while necessarily condensed, is very practical. In the discussion of the temperate aquarium, the author includes suitable aquatic plants as well as a wide variety of fish, snails and shrimps, and aquatic insects. A chapter on pond hunting describes and illustrates a variety of nets and traps useful in stocking the temperate aquarium with organisms ranging from hydra and minute crustaceans to larger forms. Much of this information is of practical value both to the amateur aquarist and to the teacher in maintaining school aquaria. Most of the organisms discussed are available in local ponds and streams. Several chapters are devoted to fresh water tropical plants and fish, including egg-layers, livebearers, bubble nesters, and cichlids. The marine aquarium and special problems relating to it are discussed in several chapters. These chapters suggest suitable small sea fish as well as anemones, marine crustacea, worms, and mollusks. The appendix contains a valuable table of exotic fish with their temperature requirements, size at maturity, origin, and pertinent information about each, in addition to a list of useful books for the aquarist. Illustrations include eight pages of photographs and numerous excellent line drawings prepared by L. R. Brightwell.

James H. Otto George Washington High School Indianapolis, Indiana

THE AMERICAN ARBACIA AND OTHER SEA UR-CHINS, 298 pp., \$6.00, Princeton University Press, Princeton, New Jersey, 1956.

This should be of considerable interest to biologists of the East Coast where these organisms are abundant enough to be the scource of considerable teacher, class, and individual student investigations. If this occurs, this book will be the source of considerable information concerning these interesting experimental animals. Also, those interested in embryology investigations will want the book. The author does not interpret the vast amount of research results accumulated, but the illustrations and reports are interesting in their own right.

M. B.

ANTS, William Morton Wheeler, 663 pp., \$17.50, Columbia University Press, New York, 1910 (Third printing, 1960).

This is the classic book on ants which has long been out of print and only rarely available. Despite its age, it is still a mine of information for the student and teacher, and much of the material covered is not available in comprehensive form anywhere else in print.

William Morton Wheeler was one of those rare scientists who was also literate. He was adept at making obscure and complicated subjects comprehensible. The approach in the present work is essentially ecological, although Wheeler sometimes expressed contempt for the narrowed applications of ecology as scientific natural history and was preeminently successful in presenting the biology of the social insects from this point of view.

For the student who wishes to continue research on ants, Wheeler's book needs to be supplemented by more recent works. Fortunately, there are now available several excellent books on the taxonomy of ants. The general behavior or ethology of ants has now been comprehensively reviewed in recent years to my knowledge, but there are now several general textbooks which will allow those interested to update Wheeler's terminology. His basic conclusions seem to me to be useful even after fifty years.

From one point of view "Ants" is completely useless. I cannot find one reference to DNA in the entire xxv + 663 pp. Ants, however, have many other attributes besides their often embarrassing ability to make and use DNA. Even if we cannot now accept without reservations all of Wheeler's evidence for ratiocination in ants, they possess many wonderful attributes. While doing a variety of other things some hold slaves, raise gardens, domesticate other animals, make use of a wide variety of chemical materials and even wage war of a sort. Fire, the wheel, and bows and arrows are not yet known among them, but don't bet on it.

Frank N. Young Department of Zoology Indiana University Y.

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MAN AND DOLPHIN, John C. Lilly, 312 pp., \$4.95, Doubleday and Company, Inc., Garden City, New York, 1961.

"Within the next decade or two the human species will establish communication with another species: nonhuman, alien, possibly extraterrestrial, more probably marine; but definitely highly intelligent, perhaps even intellectual," says John Lilly. His principal nominee for the nonhuman species is, of course, the dolphin or

porpoise.

There is no question that dolphins are clever, adaptable, and even friendly to man. After reading Lilly's accounts of his observations and experiments one is convinced that we ought to have descended from some happy, gentle, socialized creatures like these marine "playboys" rather than the skulky ape stem. The dolphin's brain is proportionately even larger and more complex than man's. There is evidence that they do communicate by means of sounds, and Lilly

also presents some fasinating evidence that they even mimic human laughter and, in a sort of short-hand form, human speech. If we are to come into communication on anything approaching an equal level, the dolphins and their relatives certainly seem to be the most likely

creatures to approach.

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Lilly, of course, is a neurophysiologist-biophysicist. His initial approach, anything but indirect, was to map the dolphin brain by the techniques already developed with dogs, monkeys, and other mammals. The first step in such operations is to drive a hypodermic needle through the skull with a hammer. The layman must wonder if this quite the proper way of going about establishing communication with a creature which may be our intellectual superior. John Lilly, however, pounded a hypodermic needle into his own skull first and found that the pain was minor, but the noise of the hammer hitting the base, reverberating through one's skull, is pretty startling. He and his coworkers have since refined their techniques and added other approaches to their attack on the problem.

In general, this is good light reading and a fair introduction to a fascinating field of research. The book suffers from several technical faults, but contains a considerable amount of sound information interlarded with the fluff.

Frank N. Young Department of Zoology Indiana University

VERTEBRATE SPECIATION, W. Frank Blair, 642 pp., \$8.50, University of Texas Press, Austin, 1961.

This is a symposium of papers read as part of the 75th Anniversary program of the University of Texas in 1958. It contains a number of valuable reviews of recent advances in the study of vertebrates and should be available to anyone teaching or carrying on research in this

general field.

One of the interesting features is the transcription of the discussions following each paper or group of papers. These often offer a pleasant diversion from the erudition of the formal presentations. The discussions are not only fun to read but often point up the purpose and the integration of the research with other fields. One feels as if he is sitting in on the real thing. One may also feel some of the ferment of modern studies of speciation. The atmosphere is a good deal more exciting than it was twenty years ago.

The program was divided into a number of sections featuring groups of papers and discussions on specific subjects. Isolating mechanisms, evolution of behavior, polymorphism, polytypic species, population dynamics, and age and origin of species are the principal sections. Each con-

tains interesting and valuable discussions not only in the form of reviews, but also new material.

Frank N. Young Department of Zoology Indiana University

Human Biology

Physiology, A Laboratory Manual, 4th Edition, Nelle A. Hartwig and Donald A. Dickmann, 169 pp., \$3.50, Burgess Publishing Company, Minneapolis, Minnesota, 1961.

This general physiology lab manual is organized around exercises on the blood, circulation, respiration, nervous system, motile tissues, digestion, and the urinary system. There are some drawings to be labeled and a great many blanks to fill after the initial exercise description. It should be examined for a possible manual in introductory courses.

P. K

ELEMENTARY PHYSIOLOGY—A LABORATORY GUIDE, Oscar E. Tauber, Robert E. Haupt, and Delma E. Harding, 190 pp., \$3.50, The Macmillan Company, New York, 1960.

Written for the elementary physiology course, the authors indicate they have kept anatomy to a minimum, but there is quite a bit of attention to this in the lab exercises. The physiology exercises are fairly orthodox, but they are well written, clear and direct, with some detail the student may need. There are many diagrams, but chiefly anatomical. The frog is the lab animal used, and there are experiments involved. Quite an adequate manual which will probably have good sales. No chemistry is assumed, but detail is given to supplement the text in all other areas.

ELEMENTARY HUMAN PHYSIOLOGY, LABORATORY AND DEMONSTRATION MANUAL, A. B. Taylor and Frederick Sargent, 99 pp., Burgess Publishing Company, Minneapolis, Minnesota, 1961.

A superlative and business-like manual for an excellent physiology course. Each of the exercises is well explained and liberally illustrated. Anatomy is gotten out of the way early in the manual with a dissection of the rat. There is no cellular physiology, but there is an abundance of other physiological work. There is a reliance on up-to-date instrumentation implied throughout. Many of the exercises lend themselves to high school use, and there is no ambiguity of instructions and directions. Of course, the physiology instructor will want to inspect this for possible use. But the high school teacher will find it a good sourcebook. Well done.

P. K.

GRAY'S ANATOMY, Henry Gray, 1458 pp., Lea & Febiger, Philadelphia, Pennsylvania, 1961.

This is the 100th year edition of this justly famous classic. The sheer size of this volume will give the user some idea of the all inclusive nature of this treatment of human anatomy. There are the familiar drawings using color. Excellent X-ray photographs are utilized to good advantage. As in previous editions, the embryology of the structures remains a very strong feature of the work. If human anatomy is the subject under study, this book remains the classic and authority.

P. K.

KRANZ MANUAL OF KINESIOLOGY, 4th Edition, Clem W. Thomson, 159 pp., \$3.75, The C. V. Mosby Company, St. Louis, Missouri, 1961.

A paperback manual written for physical education students. However, the book has a value for biology teachers interested in functional human anatomy. Each major muscle is described, and exercises both written and practical are listed. There are a series of diagrams at the end of the book to be used in laboratory work. There are no pictures, but the diagrams are excellent. This book will be useful for those biology teachers wishing to use physical education training in their teaching. Here is a sector of biology which can be used by students in biology study. Recommended book.

P. K.

HUMAN HEREDITY, Ashley Montagu, 364 pp., \$.75, The New American Library of World Literature, Inc., New York, 1960.

Another paperback of real significance. A famous anthropologist looks at the problems of human genetics in a manner not found in most texts on the subject. In the old argument between the relative importance of heredity vs. environment, Montagu takes a position of reminding those biologically oriented that environment has a tremendously important role in the production of the adult organism. He documents his case well. He is quite fair in his treatment of genetics, however, for he reviews genetic theory accurately and lucidly. This is one of the best treatments of human genetics this reviewer has seen.

An appendix lists the genetically produced abnormalties in man in a comprehensive way not found in other texts. There are chapters on radiation effects and excellent treatments of the legal status of genetics in this country. Genetic counseling receives a good treatment also.

This is an excellent book that should be on biology library shelves.

P. K.

CUTANEOUS INNERVATION, ADVANCES IN BIOLOGY OF SKIN, Vol. I, William Montagna, 203 pp., \$10.00, Pergamon Press, New York, 1960.

This volume is a formal report of the proceedings of the 1959 meeting of the symposium on The Biology of the Skin, held annually at Brown University. Edited by William Montagna, the subject of cutaneous innervation is presented from the varying points of view of the several experts in the field who contributed to the symposium. The morphology of nerve endings is presented in several well documented and excellently illustrated papers. From the functional viewpoint, the role of choline esterase is discussed, the nature of the conducting pathways, and central connections relating to cutaneous sensation are considered. The last two chapters consider the sensation of itching, analyzing the probable structures involved, possible mechanisms of action, and providing a focus which draws together, to some extent, the diverse threads of the earlier presentations.

The papers are uniformly well organized, without excessive technicality, and each chapter contains a concise summary of its salient points. Such a gathering into one volume of a large part of current knowledge about cutaneous innervation should prove a great value to clini-

cians, teachers, and experimenters.

Raymond Murray Department of Anatomy and Physiology Indiana University

HUMAN GROWTH, J. M. Tanner, 120 pp., \$5.00, Pergamon Press, New York, 1960.

The proceedings of a conference in England on the problems concerned with growth. Interesting chapters are on growth curves from various forms of data, genetics of growth, and effects of nutrition on growth. Should be of interest to those interested in adolescent growth. As in most fields of research, there are project ideas for high school students in collecting and charting growth data.

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CONCEPTS OF MEDICINE, Brandon Lush, 286 pp., \$8.50, Pergamon Press, Inc., New York, 1961.

This is a book on the practice of medicine in the form of short essays by famous physicians and scientists, most of them British. However such names as these show up: Vannevar Bush on "Professional Collaboration" and Norbert Wiener on homeostasis in medicine. The book is divided into sections on concepts of medicine, concepts of health and disease, and concepts of medical research. Most of the essays are of primary interest to physicians and medical researchers. However, the biologist will find unusual topics well discussed, such as: the meaning of normal, regulation of body temperature, life, and the evolution of the concept of disease. All these

are well written and makes one wonder as to the lost art of essay writing even though many of them started out as lectures. The book is handicapped by a lack of an index. The quotations used throughout are quite stimulating of thoughtful consideration.

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P. K.

ELECTRON MICROSCOPY IN ANATOMY, J. D. Boyd, F. R. Johnson, and J. D. Lever, Eds., viii + 288 p., \$10.00, Williams and Wilkins, Baltimore, Maryland, 1961.

This volume contains twenty papers presented in a symposium on "The Ultrastructure of Cells" at a meeting of the Anatomical Society of Great Britain and Ireland in 1958. The papers are well written and well illustrated. Most of them could be used by advanced undergraduates and graduate students in seminars dealing with electron microscopy and cytology.

John M. Hamilton Park College Parkville, Missouri

Microbiology

HANDBOOK OF BACTERIOLOGY (Mackie & McCartney's), 10th Ed., Robert Cruickshank, Ed., 979 pp., \$8.50, E and S Livingstone Limited, Edinburgh and London (Williams and Wilkins, Baltimore, Md., U. S. Agents), 1960.

This text is written, as the title page indicates, as a guide to the laboratory diagnosis and control of infection. The first section includes material normally found in the introductory chapters of any microbiology text. The second section deals with technical methods presenting a great amount of information not found in most texts published in this country. Such items as microscopy, staining techniques, sterilization, cultivation of micro-organisms, and many other aspects are presented quite well. The third section deals primarily with sixteen genera of bacteria, giving detailed information concerning morphology, culture characteristics, etc. It is within this section that we find a medical approach to the subject. Additional consideration is given to the viruses, protozoans, and molds.

The contents of this text are lucidly organized and it maintains clarity of presentation throughout. The text is primarily written for the medical student but would serve as a valuable reference

text in a biology library.

Lester Hearson Park College Parkville, Missouri

LABORATORY MANUAL FOR GENERAL MICROBIOLOGY, J. V. Beck, D. H. Larson, D. M. Donaldson, and R. D. Sagers, 56 pp., \$2.00, Burgess Publishing Company, Minneapolis, Minnesota, 1960.

This laboratory manual was planned for a one semester course in elementary microbiology having one three-hour laboratory period per week, but the experiments are so designed that the manual would be useful in other courses and in conjunction with any text. The sixteen experiments introduce the student to the use of the microscope, staining procedures, pure culture techniques, and biochemical activities of common bacteria, effects of environment, food and water bacteria, etc. Formulae for staining solutions and media are included in appendix sections, and thought provoking questions are given for each experiment. This should be a useful manual for introductory courses in bacteriology with restricted laboratory hours, especially for courses for the general, non-major, student.

> L. S. McClung Department of Bacteriology Indiana University

BIOLOGY THROUGH MICROBES—A LABORATORY GUIDE, Alfred S. Sussman, 202 pp., University of Michigan Press, Ann Arbor, Michigan, 1961.

It is hard to see how a beginning student could not help but be intrigued by the lab work which this manual unfolds. The author has taken microbiology as a method of teaching fundamental biology. Each exercise is headed by an intriguing quotation that serves to set the tone and hint at the teaching objective concerned. Each of the exercises begins with some unknowns and goes from there to attempt to lead the student to do some real scientific thinking. For instance, a strange mixture concocted by the student is used as an experimental medium to culture microorganisms under varying conditions. The real imagination the author has shown in all the exercises from bacteria, protozoa, algae, fungi, to the viruses, continues in those on metabolism, reproduction, and disease. Pop-beads are used for some biochemical understandings. A real gem of a lab guide and a model for other areas. Should be on the shelf of every teaching biologist.

LABORATORY MANUAL FOR DAIRY MICROBIOLOGY, E. M. Foster and W. C. Frazier, 72 pp., \$3.00, Burgess Publishing Company, Minneapolis, Minnesota, 1961.

The thirty experiments, some of which have been adapted for class use from the APHA Standard Methods for the Examination of Dairy Products, included in this college level laboratory manual provide a fundamental introduction to laboratory work in dairy microbiology. High

school students who wish to do projects in this field will find clear-cut instructions regarding standard tests and, in addition, an appendix which gives adequate explanation of the results of various tests. The experiments are grouped in three sections: study of milk microorganisms, methods used in the control of milk quality, and microbiology of dairy products. Questions and references are included for each experiment. This high quality manual is the result of collaboration of two well known microbiologists who have had many years of experience in one of the leading dairy research laboratories of the world.

L. S. McClung
Department of Bacteriology
Indiana University

Spores II, H. Orin Halvorson, Ed., 296 pp., \$5.00, Burgess Publishing Company, Minneapolis, 1961.

This volume consists of 27 technical papers on various aspects of microbial sporulation and germination presented at the second Spore Symposium held at the University of Illinois in October, 1960. Much of the work described is fragmentary and, as some of the authors mention, will be described more completely in other publications. Nevertheless the volume will be quite valuable to newcomers to this field because the collected papers provide an excellent survey of current problems that are being attacked, the methodology employed, and a good background of references for each problem. As with most symposia, the chief value to experienced workers probably was their participation or attendance at the symposium itself; unfortunately, any informal discussions following the papers were not included in the published material. The volume is attractively printed but suffers from a number of minor typographical errors and the lack of an index.

Eugene D. Weinberg Department of Bacteriology Indiana University

THE CELLULAR SLIME MOLDS, John Tyler Bonner, 149 pp., \$4.00, Princeton University Press, Princeton, New Jersey, 1959.

In this book the author carefully and interestingly tells us of this most fascinating group of organisms. Citations and references are complete, so that the teacher and student looking for project ideas could easily find a wealth of material for such work. Distinctions are quickly made among the aggregation organisms. Also the physiology of their motion and sporulation is discussed thoroughly. A wonderful book to read and enjoy—yet one to be used also for authoritative information on this group.

THE ENCYCLOPEDIA OF MICROSCOPY, George L. Clark, Ed. xiii + 693 p., \$25.00, Reinhold Publishing Corp. New York, 1961.

A scientist not only must understand the instruments he uses in his research, but also he must have a knowledge of the theory and application of many instruments he never uses in order to be able to read the literature intelligently and recognize the limitations and the possibilities of the instruments used by others, The Encyclopedia of Microscopy should help to provide this knowledge. It consists of over 140 articles by an international panel of authors. The majority of these treat the use of the microscope in physical science and industry, but one should not conclude that there is little in the book for the biologist. In addition to valuable articles on theory and methods of microscopy, there are numerous sections dealing with purely biological subjects. In the section on electron microscopy, for example, there are articles on blood; botanical applications; cell ultrastructure in mammals; ciliated epithelium ultrastructure; kidney ultrastructure; leaf surfaces; kidney pathology; and connective tissue, bones, and teeth.

The book is generally well written and well illustrated. References are given at the end of most of the articles. There is some cross-indexing, but unfortunately there is no general

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John M. Hamilton Park College Parkville, Missouri t

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Oncogenic Viruses (International Series of Monographs on Pure and Applied Biology, vol. 2), Ludwik Gross, 385 pp., \$12.00, Pergamon Press Ltd., New York, 1961.

This book is concerned with the summation and valuation of the evidence indicating that viruses are the causative agent in certain animal cancers. Obviously a main objective of the book is evaluation by analogy of the extrapolation of these data to the idea that viruses cause many, or conceivably all, human cancers. Anyone who is a teacher or who lectures occasionally finds that the public is fascinated by the idea of cancer being caused by viruses. Additionally, among professional virologists the question of the general viral etiology of cancer is one which is hotly debated. The book is, therefore, timely. In part it is technical, but the scope and presentation is such that not only virologists but laymen, including students, should find much of it fascinating.

Each section of *Oncogenic Viruses* includes historical material and, in those instances where it has been possible, Gross has corresponded with the discoverer of the phenomenon in

question and is able to present otherwise quite unavailable personal sidelights on the origins of the work. The first and last chapters of the book are of a general nature and should be read by everyone, since they present very succinctly the present position with regard to the etiology of cancer. In addition, there are special sections on all (some twenty) of the cancer viruses of any importance; there is much of interest to the general reader in every chapter. A wealth of pictures of experimenters and illustrations of cancerous animal materials adds to the attractiveness.

To the professional virologist, Oncogenic Viruses appears to be an admirably well-written, modern, and carefully documented presentation of the current state of an important topic. In many instances, again, the author has interpolated -obviously in proof—the most recent results or statements derived from personal communications. The book presents, in most instances, a summary of the actual data in important experiments plus, where deemed desirable by the author, evaluation, e.g., whether the animals used were of well-characterized strains, whether the work has been repeated, etc.

In some respects, the reviewer found Oncogenic Viruses a disappointment. For the non-medical specialist, even, a glossary of the more medical terms would have been a help. Also, nowhere has the author included pictures of control animals or tissue sections so that the non-expert could make a comparison with the experimental material. The areas of interest in the pictures might easily, for example, have been identified by arrows; instead they are designated only descriptively, e.g., (Fig. 20) "Large thymic tumor . . . large tumorous lymph nodes in the

mesentery."

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A more serious criticism concerns the sections on mouse leukemia and the polyoma virus. These chapters are excessively detailed and disproportionately long. One has the feeling that the author has lost his sense of proportion in handling those subjects with which he is personally concerned a not uncommon failing. But the object of this lengthy treatment seems to be entirely an effort to establish the author as the discoverer of the most important polyoma virus. Not only does he play down the work of Stewart and Eddy at every turn, but he insistently endeavors to indicate that their generally accepted work is only an elaboration of his own. The reviewer is not competent to judge this polemic, but it is at the least in questionable taste and grossly overburdened with detail.

As a bacterial virologist, the reviewer would like to take issue with several negative generalizations about viruses, e.g., (p. 6) "What happens in the cell following the entry of a virus is poorly

understood."; (p. 334) "Our knowledge of the physical and chemical composition of virus particles is only fragmentary." Perhaps the reviewer and author are both being a little parochial, however.

On the whole the book is admirable. It is up-to-date, well written, profusely illustrated, and an authoritative account of a most important area of medical research. The number of footnotes and references is enormous. Oncogenic Viruses would deserve a place in any technical library as a reference work. But, in addition, because of its importance, scope, and interesting presentation, it should also be in libraries designed for the general use of students and the reading public.

Dean Fraser Department of Bacteriology Indiana University

Growth and Development

How Life Began, Irving Adler, 128 pp., \$.35, New American Library of World Literature, Inc., New York, 1957.

Another paperback which belies its title in the sense that about half the book is devoted to elementary chemistry which goes easily into an interesting account of some biochemistry. The book begins with an account, somewhat historical, of the difference between life and death. There are some accounts of cycles, and there are interesting implications of them in their chemical relationships without being pedantic. An interesting little paperback especially for those wanting an elementary biochemical background to biology.

P. K

The Atoms Within Us, Ernest Borek, 272 pp., \$5.00, Columbia University Press, New York, 1961.

This is another popular account of the role biochemistry has played in the understanding of biology. No chemical formulae are used, and the author has an easy, readable style which will make the book a useful one to recommend for student reading. The reviewer is puzzled by the reference in the preface to avoiding the names of prominent scientists in talking about the amazing discoveries of the "molecular biologist." However, a great many names are cited, and it is hard to see how their mention can be avoided.

While at first glance the book seems organized around important topics in this science, a historical approach is obvious on reading. Headings such as sugars, vitamins, and blood are common as chapter titles, and this will give some idea of the scope of the treatment.

The author is unafraid of tackling controver-

sial issues and editorializing on them. The last chapter has a plea for public understanding of the financial support necessary for science, and there is at least one pointed remark against antivivisectionists.

It is a good book for the school general library, but it can be recommended for teacher and student in gaining some understanding of this phase of biology.

P. K.

THE LIFE AND DEATH OF CELLS, Joseph G. Hoffman, 354 pp., \$.95, Dolphin Books, Doubleday and Company, Inc., Garden City, New York, 1957.

A biophysical slant on cytology is given in this paperback. It is written in an easy manner with a minimum of complex tables, etc. In fact, there is no mathematics used, but illustrations might have been used more frequently to illustrate some of the very important points. It seems obvious that the author is especially interested in cancer research because a great deal of interesting facts are given with some reference to the researcher involved. However, some of the other big ideas in this field are presented well but with no reference to the men concerned.

The book seems especially good in discussing radiation effects, tissue culture, experimental embryology, and cancer. There are interesting chapters on some of the biological implications of protein synthesis, electrical nature of protoplasm, and DNA. This book will be fine for the reader wanting some clearly stated background on modern research on the cell. But time flies, and some references are already out of date, such as the human chromosome number.

P. K.

GROWTH, DEVELOPMENT, AND PATTERN, N. J. Berrill, 555 pp., \$10.00, W. H. Freeman and Company, San Francisco, California, 1961.

This book is a compilation of descriptive and experimental studies on morphogenesis and an attempt to formulate some general hypotheses. The emphasis is on growth and asexual reproduction in the coelenterates and tunicates which the author has utilized extensively in his own research. It is thus highly personal and selective, making no claims to completeness. At the same time, however, the wide range of Dr. Berrill's interests and knowledge, and his exceedingly complete presentation of some of the material, make the volume useful as a reference source. There is a good deal of information about sponges (reaggregation), annelid worms (regeneration), other invertebrates, vertebrate embryology, and even a section of the plant meristem. The descriptions and discussion are for the most part given in terms of cells and tissues, and there is

very little mention of biochemical approaches to the problems. The general hypotheses which are proposed deal with such matters as the relation between growth rate and differentiation, the interaction of adjacent "morphogenetic fields," and similar phenomena. Admittedly tentative and preliminary, these hypotheses will not be accepted by everyone but should stimulate a good deal of thought and discussion.

Critical reading of this book requires some prior knowledge of embryology and invertebrate zoology; it will thus be directly useful primarily to students at the graduate level and to the more advanced undergraduate. High school and college teachers will find it a valuable source of material to be presented to their students after suitable condensation and simplification. The book is beautifully and thoroughly illustrated and for the most part clearly written. The bibliography includes references up to 1959; the index is, unfortunately, rather scanty. The reviewer recommends that readers glance at the hypotheses, lumped together in Chapter 15, before reading the first 14 chapters. A brief statement of these hypotheses at the beginning of the book would have helped the reader to evaluate them as he reads the descriptive material upon which they are based.

C. W. Birky, Jr. Department of Zoology Indiana University B

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CARCINOGENESIS BY ULTRAVIOLET LIGHT, Harold F. Blum, 340 pp., \$6.50, Princeton University Press, Princeton, New Jersey, 1959.

The author has provided us another classic in scientific research reports which goes beyond professional science writing and still is not a highly formalized research report capsulized in brief form. The title is self-explanatory for this book traces the idea of carcinogenesis by ultraviolet light through preliminary ideas, research results, and finally to an elaborately reasoned conclusion. This book is far beyond the title however. The author is a skilled writer, as well as a researcher, and his descriptions of the process of carcinogenesis and all its ramifications are skillfully handled. The book illustrates how all the resources of the biologist have to be used to bear on what seems to be a rather narrow problem. Ultraviolet light and quantum theory have to be examined. Then a resume of the studies on the effects of ultraviolet light on cells are recounted. Cell growth and developmental changes are charted.

All in all, this is a book to be recommended to the serious student to show the care involved in a research project. However, the teacher would also profit by its reading.

P. K.

Index for Volume 23

Compiled by John D. Woolever Riverview High School, Sarasota, Florida

AUTHORS

Alexander, Von C., Experimental Course in Field Biology for Superior High School Students, January, pp. 36-37.

Bennett, Jack, A Simplified Method of Drosophila Culture for the Classroom, February, pp. 79-82. Beste, Vernance, Techniques for Developing Interests in Senior High School Biology, March, pp. 143-146.

Breukelman, John, Writing Articles for Publication,

October, pp. 354-364.

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Brown, Randolph R., Homeostasis and Kidney Functions, October, pp. 355-358. Burlington, Roy F. and John Mark Dean, Study of

the Fresh Water Stream, May, pp. 291-293. Butterfield, Charles H., The Biology Classroom Ex-

tended, March, pp. 141-142.

Chan, Gordon, An Ancient Method of Fish Mount-

ing, November, pp. 436-438. Charles, Brother H., Biology in the News, February, p. 82. March, p. 142. November, p. 435.

Charney, Sol, A Method for Recent Proposals, March, pp. 135-136.

Cicero, Donald and John Gabriel Navarra, Experimenting With Planaria, December, pp. 516-518. Cornwell, George Wm. and Ernest G. Kirby, Conservation Education in Action, April, pp. 210-213. Crombie, Charles, Building a Terrarium, January, p.

Crooks, Kenneth B. M., Suggestions for Teaching the Scientific Method, March, pp. 154-159.

Davis, John D., Physiological Demonstration of the Quahog Heart: Apparatus and Techniques, November, pp. 439-442.

Dean, John Mark, and Roy F. Burlington, Study of the Fresh Water Stream, May, pp. 291-293. Dindal, Daniel Lee, Ecology Studies in High School

Biology, May, pp. 281-284.

Eddleman, Harold L., Genetics Experimentation in High School Biology, February, pp. 83-89.

Editor, AAAS Botany Symposium, November, p. 448. ABT Regional Directors, May, pp. 293-294. Aerial Spraying, December, p. 522.

African Education, November, p. 435. AIBS Grant, December, p. 512

AIBS Meeting, February, p. 91. AlBS Visiting Biologists Program, December, p.

1961 AIBS Meeting, March, p. 164.

Air Attack on Forest Fires, March, p. 138.

Air Force Needs Teachers for Overseas School Program, April, p. 218.

Air Force Overseas Dependent School Program

1961-1962, February, p. 95. Allergy, March, p. 142. American Society for Microbiology, April, p. 204. Atomic Commission's Report, February, p. 89. Bacteriology Manual, March, p. 150.

Bard Award, November, p. 442. Biochemistry, October, p. 354.

Biological Control, April, p. 209. Biology Students in India, October, pp. 364-366.

BSCS Films, March, p. 138.

Cancer, May, p. 280. Career Publication, January, p. 37. Century 21 Exposition, October, p. 354.

Cholesterol Relative, May, p. 275. College Botany Conference, May, p. 287.

College Scholarships, October, p. 347. Coming Issues of ABT, March, p. 159.

Conservation, February, p. 78. The Curious Naturalist, May, p. 275. Depression Drugs, February, p. 106.

Detection, October, p. 347.

Discovery of Fish Remains May Support 50-Year-Old Antarctic Ice Shelf Theory, April, p. 204.

Doctors Smoking Less, May, p. 290. Drosophila Bulletin, April, p. 204. Encouraging Writing, November, p. 438.

Endeavour Prizes, May, p. 275.

European Travel Study Program in Comparative Education, February, p. 78.

Fact, October, p. 354. Facts About Strokes, February, p. 98.

Fallout, May, p. 272. Fellowships, January, p. 37.

Film Bibliography, March, p. 140. Film Catalog Supplement, May, p. 275.

Film Review, February, p. 95.

Fluorescence Microscopy Aiding Medicine and Chemistry, April, p. 221.

Food Additives, October, p. 354. Foreign Service Posts, March, p. 146.

Gas Chromatography, February, p. 103. Geographic School Bulletin, December, p. 505. Gradwohl Memorial Scholarship, November, p.

Hallucinations, May, p. 284.

Heart Stimulant, April, p. 226. History of Wild Life Research, January, p. 41. How to Donate Your Body for Medical Science, October, p. 347

Job Futures for Girls in Biology, October, p. 347.

John Hays Fellows, December, p. 519. Junior Scientists Transactions, November, p. 435.

Lab Equipment, November, p. 448.

Leukemia, May, p. 287. Living Insects and Mites Found on High Antarctic Plateau, October, p. 351.

Local Periodical, March, p. 140.

Local Science Meeting, December, p. 512.

Mark, May, p. 275. Measures and Weights, February, p. 76.

Memory, March, p. 136. Microbiology in Your Future, November, p. 448. NABT Teacher Award Program, December, p. 515. NABT and NEBA Combined Meeting, October, p. 347.

NESSAC, February, p. 89.

New Bibliography, January, p. 17.

New NABT Officers, January, p. 40.

Ninth Paul B. Mann Congress, May, p. 272. Nominations for 1962 Officers, November, pp. 449-

NSF Graduate Fellowships, December, p. 519.

NSF Publication, March, p. 153.

NSTA Annual Meeting, March, p. 138.

Pamphlet, January, p. 28. Paragraphs To Learn By, November, p. 448.

Physical Facilities, February, p. 82.

Positions for Teaching Assistant in Conservation

Education, May, p. 280.

Post-Doctoral Fellowships, December, p. 512. Proposed Revisions of the Constitution of NABT,

October, pp. 346-347.

Purchase Guide Supplement, January, p. 45. Room for Young Scientists, February, p. 95.

Science Activities Bulletin, January, p. 22. Science Fair Winners, February, p. 93.

Science Student Teachers, October, p. 347.

Scientists Complete 65-Day Trek to South Pole, October, p. 351.

Sea Lamprey, April, p. 218.

Senior Visiting Science Fellowships for OEEC Awarded by NSF, April, p. 221.

Sierra Science Journal, October, p. 354.

Skin Cancer, January, p. 45.

State Meeting, March, p. 146. Student Research, March, p. 167.

Summer Graduate Courses in Botany, December, p.

Teachers Wanted Abroad, February, p. 89.

Tree Pamphlets, April, p. 218.

Tribolium Guide, January, p. 26.

Trigger, January, p. 35.

US Scientists Begin Cooperative Antarctic Research Program With Chilean Expedition, April, p. 209.

Virus Reproduction, February, p. 76.

"Vistas of Science," December, p. 512. Water Pollution Conference, May, p. 275.

Women in Biology Leaflet, December, p. 512. Evans, Everett F., Conservation Activities in High School Biology, April, pp. 222-225.

Fagle, David L., Teaching Microbiology in High School Biology, January, pp. 42-45.

Feiro, Arthur D., Presenting-The Cellular Slime Molds, December, pp. 501-505.

Fowler, H. Seymour, AAAS Cooperative Committee Meeting, November, p. 443.

Fox, Richard, A.V. News, May, pp. 294-296; December, pp. 499-500.

Frankel, Edward, An Advanced Studies Program in Biology, January, pp. 18-22.

Glass, Arthur W., A Review Method for Courses in Biology, March, pp. 137-138.

Graves, Artis P., Use of Amphibians in Advanced Embryology Classes, February, pp. 99-103.

Harper, Dennis, and Jerry Levy, On the Origin of Organic Compounds, October, pp. 348-351.

Haves, Richard K., Advanced Biology in Summer School, January, pp. 27-28.

Hilton, William A., The Importance of Biological

Research, January, pp. 38-39. Holmquist, A. M., Field Studies Brought to the

Laboratory, April, pp. 225-226. Hoshaw, Robert W., Sexual Cycles of Three Green Algae For Laboratory Study, December, pp. 489-

Katz, Lillian, and John Gabriel Navarra, Seed Extracts and Human Blood Typing, February, pp. 92-93.

Keefe, Rev. Anselm M., O. Praem, Demonstrating Sap-Rise, February, pp. 94-95.

Kirby, Ernest G., and George Wm. Cornwell, Conservation Education in Action, April, pp. 210-213. Klotz, John W., An Experiment in the Scientific Method, March, pp. 165-167.

Lee, Addison E., An Introduction to the BSCS Lab-oratory Block Program, November, pp. 409-411. Plant Growth and Development, November, pp.

Levy, Jerry, and Dennis Harper, On the Origin of

Organic Compounds, October, pp. 348-351. Liebherr, Harold G., Field Biology in the High School Program, May, pp. 285-287.

Lightner, Jerry P., A Report on the Status of Advanced Biology in Large Secondary Schools of the United States, January, pp. 7-17.

Lisonbee, Lorenzo, Mt. Lemmon Revisited, May, pp. 288-290.

Litweiler, Ernest, Experimental Approach to the Study of Fresh Water Organisms, December, pp. 520-522.

Lokke, Donald H., Cloud Observations Applied to Ecological Studies, April, pp. 208-209.

Manning, Wayne E., Have You Used Phluoroglucin Solution? December, pp. 518-519.

Marx, Thomas I., Construction and Use of a Simple Ophthalmoscope, February, pp. 71-76. Miller, Arnold I., Studying the Effect of Various

Crude Drug Media on the Cultivation of Fungi, February, pp. 77-78.

Miller, William H., Root Tip Cell Squashes for the Study of Cell and Chromosome Morphology, December, pp. 513-515.

Moog, Florence, Animal Growth and Development, November, pp. 418-423.

Moser, Gene W., The Use of Simocephalus Vetulus Embryos in the Teaching of Embryology, February, pp. 96-98.

Muller, H. J., Life Forms to Be Expected Elsewhere than on Earth, October, pp. 331-346.

Munzer, Martha E., Science and Natural Resources-A Different Filmstrip Approach, April, pp. 219-

V

A

Navarra, John Gabriel and Donald R. Cicero, Experimenting With Planaria, December, pp. 516-518.

Navarra John Gabi el, and Lillian Katz, Seed Extracts and Humar Blood Typing, February, pp. 92-93.

Nicolai, F. L., The Application of Inductive Procedures to Selected Topics for High School Biology, March, pp. 151-153.

Overmire, Thomas G., The 6th Sense-Nonsense?, March, pp. 139-140.

Palmer, E. Lawrence, The International Union for the Conservation of Nature and Natural Resources, April, pp. 227-234. Parin, Vassily, The Cycle of Nature in the Space-

ship Cabin, October, pp. 352-353. Patterson, Florence K., A Tree Game for Fun in

ical

the

een

189-

Ex-

pp.

ting

on-

213.

rific

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11,

pp.

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Ad-

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May,

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Feb-

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es-

219-

peri-

Ex-

pp.

Pro-

l Bi-

ense?,

Biology, April, pp. 217-218. Pierson, David W., A Biology Class Project Illustrating Wind Erosion, April, pp. 205-208.

Regan, James D., Radiation Studies with Neurospora crassa, February, pp. 90-91.

Richards, A. Glenn, The Interdependence of Struc-

ture and Function, November, pp. 423-429. Roth, Charles E., Field Studies for High School Biology Students, April, pp. 213-217.

Scheel, Leonard G., Why Follow the Leader? May, pp. 276-278.

Seneca, Arlena E., Whack That Quack-Report of a Classroom Project that Involved the Community, March, pp. 147-150.

Sherman, Kenneth, High School Oceanographic Laboratory, January, pp. 29-35.

Unique Growth Pattern for Laboratory Observa-

tion, February, pp. 104-106. Shough, W. W., High School Class in Ecology, May, pp. 279-280. Snyder, C. Richard, Effects of Radiation on Germi-

nation Patterns of Spores of Certain Mosses and Ferns, December, pp. 506-512.

Steere, William Campbell, Biological Problems in Arctic America, May, pp. 263-272.

Stopyra, Ted, Polarization of Cells and Crystals, December, pp. 523-524.

Sund, Robert B., The Use of Bottled Blood in the Classroom, February, pp. 107-108.

Sussman, Alfred S., Microbes: Their Growth, Nutrition, and Interaction, November, pp. 411-418. Syrocki, B. John, Experimenting with Hydroponics, November, pp. 444-448.

Tignor, Donald M., The Scientific Method-Another

Look, March, pp. 160-164. Turner, George C., Outline for a High School Second Year Biology Course, January, pp. 23-26.

Vologdin, A., Tracing the Origin of Life, October, pp. 353-354.

Weaver, Howard E., The Countdown, April, pp.

Wise, Charles D., Bathtub Ecology for the Biology Class, February, pp. 108-109.

Wolf, Frank E., Acid plus Base → Salt plus Water, January, p. 40.

The Response of Paramecia to Electricity, January,

Woolever, John, Index for Volume 23, December, pp. 535-545.

Young, Darrell D., Dissection of the Common House Fly, December, pp. 524-525.

Youngpeter, John M., Field Trips, May, pp. 273-275.

TITLES

AAAS Botany Symposium, Editor, November, p. 448.

AAAS Cooperative Committee Meeting, H. Seymour Fowler, November, p. 443.

ABT Regional Directors, Editor, May, pp. 293-294. Acid plus Base-Salt plus Water, Frank E. Wolf,

January, p. 40.
Advanced Biology in Summer School, Richard K.

Hayes, January, pp. 27-28. Advanced Studies Program in Biology, An, Edward Frankel, January, pp. 18-22.

Aerial Spraying, Editor, December, p. 522. African Education, Editor, November, p. 435.

AIBS Grant, Editor, December, p. 512. AIBS Meeting, Editor, February, p. 41. AIBS Meeting, Editor, March, p. 164.

AIBS Visiting Biologist Program, Editor, p. 505. Air Attack on Forest Fires, Editor, March, p. 138. Air Force Needs Teachers for Overseas School Program, Editor, April, 1961, p. 218.

Air Force Overseas Dependent School Program 1961-1962, Editor, February, p. 95.

Allergy, Editor, March, p. 142.

American Society for Microbiology, Editor, April, p. 204.

Ancient Method of Fish Mounting, An, Gordon Chan, November, pp. 436-438.

Animal Growth and Development, Florence Moog,

November, pp. 418-423.

Application of Inductive Procedures to Selected Topics For High School Biology, The, F. L. Nicolai, March, pp. 151-153.

Atomic Energy Commission's Report, Editor, February, p. 84. AV News, Richard Fox, May, pp. 294-296; December,

pp. 499-500.

Bacteriology Manual, Editor, March, p. 150. Bard Award, Editor, November, p. 442.

Bathtub Ecology for the Biology Class, Charles D. Wise, February, pp. 108-109.

Biochemistry, Editor, October, p. 354.

Biological Control, Editor, April, p. 209. Biological Problems in Arctic America,

Campbell Steere, May, pp. 263-272.

Biology Classroom Extended, The, Charles H. Butterfield, March, pp. 141-142.

Biology Class Project Illustrating Wind Erosion, A, David W. Pierson, April, pp. 205-208.

Biology In The News, Brother H. Charles, February, p. 82; March, p. 142; November, p. 435.

Biology Students in India, Editor, October, pp. 364-

BSCS Films, Editor, March, p. 138.

Building a Terrarium, Charles Crombie, January, p. 41.

Cancer, Editor, May, p. 280. Career Publication, Editor, January, p. 37. "Century 21 Exposition," Editor, October, p. 354.

Cholesterol Relative, Editor, May, p. 275. Cloud Observations Applied to Ecological Studies, Donald H. Lokke, April, pp. 208-209.

College Botany Conference, Editor, May, p. 287. College Scholarships, Editor, October, p. 347. Coming Issues of ABT, Editor, March, p. 159.

Conservation, Editor, February, p. 78.

Conservation Activities in High School Biology, Everett F. Evans, April, pp. 222-225.

Conservation Education in Action, George Wm. Cornwell and Ernest G. Kirby, April, pp. 210-213. Construction and Use of a Simple Ophthalmoscope, Thomas I. Marx, February, pp. 71-76. Countdown, The, Howard E. Weaver, April, pp.

199-204.

Curious Naturalist, Editor, May, p. 275.

Cycle of Nature in the Spaceship Cabin, The, Vassily Parin, October, pp. 352-353.

Demonstrating Sap-Rise, Rev. Anselm M. Keefe, O. Praem, February, pp. 94-95.
Depression Drugs, Editor, February, p. 106.

Detection, Editor, October, p. 347.

Discovery of Fish Remains May Support 50-Year-Old Antarctic Ice Shelf Theory, Editor, April, p. 204.

Dissection of the Common House Fly, Darrell D. Young, December, pp. 524-525.

Doctors Smoking Less, Editor, May, p. 290. Drosophila Bulletin, Editor, April, p. 204.

Ecology Studies in High School Biology, Daniel Lee Dindal, May, pp. 281-284.

Effects of Radiation on Germination Patterns of Spores of Certain Mosses and Ferns, C. Richard Snyder, December, pp. 506-512

Encouraging Writing, Editor, November, p. 438.

Endeavour Prizes, Editor, May, p. 275.

Epileptics, Editor, May, p. 284. European Travel Study Program in Comparative Education, Editor, February, p. 78.

Experiment in the Scientific Method, An, John W.

Klotz, March, pp. 165-167. Experimental Approach to the Study of Fresh Water

Organisms, Ernest Litweiler, December, pp. 520-

Experimental Course in Field Biology for Superior High School Students, An, Von C. Alexander, January, pp. 36-37

Experimenting with Hydroponics, B. John Syrocki, November, pp. 444-448.

Experimenting With Planaria, John Gabriel Navarra and Donald R. Cicero, December, pp. 516-518.

Fact, Editor, October, p. 354.

Facts about Strokes, Editor, February, p. 98.

Fallout, Editor, May, p. 272.

Fellowships, Editor, January, p. 37

Field Biology in the High School Program, Harold

G. Liebherr, May, pp. 285-287. Field Studies Brought to the Laboratory, A. M.

Holmquist, April, pp. 225-226. Field Studies for High School Biology Students,

Charles E. Roth, April, pp. 213-217. Field Trips, John M. Youngpeter, May, pp. 273-275. Film Bibliography, Editor, March, p. 140.

Film Catalog Supplement, Editor, May, p. 275.

Film Review, Editor, February, p. 95. Filmstrips (Geology and Soil-Series of Five),

Marian Ray, May, pp. 296-297. horescence Microscopy Aiding Medicine and Fluorescence Chemistry, Editor, April, p. 221.

Food Additives, Editor, October, p. 354. Foreign Service Posts, Editor, March, p. 146.

Gas Chromatography, Editor, February, p. 103. Genetics Experimentation in High School Biology, Harold L. Eddleman, February, pp. 83-89.

Geographic School Bulletin, Editor, December, p. 505. Gradwohl Memorial Scholarship, Editor, November,

Hallucinations, Editor, May, p. 284.

Have You Used Phluoroglucin Solution? Wayne E. Manning, December, pp. 518-519.

Heart Stimulant, Editor, April, p. 226.

High School Class in Ecology, A, W. W. Shough, May, pp. 279-280. High School Oceanographic Laboratory, Kenneth

Sherman, January, pp. 29-35. History of Wild Life Research, Editor, January,

p. 41.

Homeostasis and Kidney Functions, Randolph R. Brown, October, pp. 355-358.

How to Donate Your Body for Medical Science, Editor, October, p. 347.

P

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611

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Importance of Biological Research, The, William A. Hilton, January, pp. 38-39.

Index for Volume 23, John D. Woolever, December, pp. 535-545.

Interdependence of Structure and Function, The, A. Glenn Richards, November, pp. 423-429. International Union for the Conservation of Nature

and Natural Resources, The, E. Laurence Palmer, April, pp. 227-234.

Introduction to the BSCS Laboratory Block Program, An, Addison E. Lee, November, pp. 409-411.

Job Futures for Girls in Biology, Editor, October, John Hays Fellows, Editor, December, p. 519.

Junior Scientists Transactions, Editor, November, p.

Lab Equipment, Editor, November, p. 448. Leukemia, Editor, May, p. 287.

Life Forms to Be Expected Elsewhere Than on Earth,

H. J. Muller, October, pp. 331-346, Living Insects and Mites Found on High Amarctic, Editor, October, p. 351.

Local Periodical, Editor, March, p. 140. Local Science Meeting, Editor, December, p. 512.

Mark, Editor, May, p. 275.

Measures and Weights, Editor, February, p. 76. Memory, Editor, March, p. 136.

Method for Recent Proposals, A, Sol Charney, March, pp. 135-136.

Microbes, Their Growth, Nutrition, and Interaction,

Alfred S. Sussman, November, pp. 411-418. Microbiology in Your Future, Editor, November, p. 448.

Mt. Lemmon Revisited, Lorenzo Lisonbee, May, pp. 288-290.

NABT Teacher Award Program, Editor, December, p. 515.

NABT and NEBA Combined Meeting, Editor, October, p. 347. NESSAC, Editor, February, p. 89.

New Bibliography, Editor, January, p. 17. New NABT Officers, Editor, January, p. 40.

Ninth Paul B. Mann Congress, Editor, May, p. 272. Nominations for 1962 Officers, Editor, November,

pp. 449-455. NSF Graduate Fellowships, Editor, December, p. 519.

NSF Publication, Editor, March, p. 153. NSTA Annual Meeting, Editor, March, p. 138.

On the Origin of Organic Compounds, Dennis Harper and Jerry Levy, October, pp. 348-351. Outline for a High School Second Year Biology

Course, George C. Turner, January, pp. 23-26.

Pamphlet, Editor, January, p. 28.

Paragraphs To Learn By, Editor, November, p. 448.

Physical Facilities, Editor, February, p. 82. Physiological Demonstration of the Quahog Heart: Apparatus and Techniques, John D. Davis, November, pp. 439-442.

Plant Growth and Development, Addison E. Lee,

November, pp. 430-435. Polarization of Cells and Crystals, Ted Stopyra, De-

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272.

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, p.

cember, pp. 523-524.
Position for Teaching Assistant in Conservation Education, Editor, May, p. 280.

Post-Doctoral Fellowships, Editor, December, p. 512. Presenting-The Cellular Slime Molds, Arthur D. Feiro, December, pp. 501-505.

Proposed Revisions of the Constitution of NABT,

Editor, October, pp. 346-347. Purchase Guide Supplement, Editor, January, p. 45.

Radiation Studies with Neurospora crassa, James D.

Regan, February, pp. 90-91.

Report on the Status of Advanced Biology in Large Secondary Schools of the United States, A, Jerry P. Lightner, January, pp. 7-17. Response of Paramecia to Electricity, The, Frank E.

Wolf, January, p. 22. Review Method for Courses in Biology, A, Arthur

W. Glass, March, pp. 137-138.

Room for Young Scientists, Editor, February, p. 95. Root Tip Cell Squashes for the Study of Cell and Chromosome Morphology, William H. Miller, December, pp. 513-515.

Science Activities Bulletin, Editor, January, p. 22. Science Fair Winners, Editor, February, p. 93.

Science and Natural Resources—A Different Film-strip Approach, Martha E. Munzer, April, pp. 219-

Science Student Teachers, Editor, October, p. 347. Scientific Method-Another Look, The, Donald M. Tignor, March, pp. 160-164.

Scientists Complete 65-Day Trek to South Pole, Editor, October, p. 351.

Sea Lamprey, Editor, April, p. 218.

Seed Extracts and Human Blood Typing, John Gabriel Navarra and Lillian Katz, February, pp.

Senior Visiting Science Fellowships for OEEC Awarded by NSF, Editor, April, p. 221.

Sexual Cycles of Three Green Algae For Laboratory Study, Robert W. Hoshaw, December, pp. 489-499.

Sierra Science Journal, Editor, October, p. 354. Simplified Method of Drosophila Culture for the Classroom, A, Jack Bennett, February, pp. 78-82. 6th Sense-Nonsense, The, Thomas G. Overmire, March, pp. 139-140.

Skin Cancer, Editor, January, p. 45.

State Meeting, Editor, March, p. 146.

Student Research, Editor, March, p. 167. Study of the Fresh Water Stream, A, Roy F. Burlington and John Mark Dean, May, pp. 291-293.

Studying the Effect of Various Crude Drug Media on the Cultivation of Fungi, Arnold I. Miller, February, pp. 77-78.
Suggestions for Teaching the Scientific Method,

Kenneth B. M. Crooks, March, pp. 154-159. Summer Graduate Courses in Botany, Editor, Decem-

ber, p. 505.

Teachers Wanted Abroad, Editor, February, p. 89. Teaching Microbiology in High School Biology,

David L. Fagle, January, pp. 42-45. Techniques for Developing Interests in Senior High School Biology, Vernance Beste, March, pp. 143-

Tracing the Origin of Life, A. Vologdin, October, pp. 353-354.

Tree Game for Fun in Biology, A, Florence K. Patterson, April, pp. 217-218.

Tree Pamphlets, Editor, April, p. 218. Tribolium Guide, Editor, January, p. 26.

Trigger, Editor, January, p. 35.

Unique Growth Pattern for Laboratory Observation, A, Kenneth Sherman, February, pp. 104-106. Use of Amphibians in Advanced Embryology Classes,

Artis P. Graves, February, pp. 99-103. Use of Bottled Blood in the Classroom, The, Robert

B. Sund, February, pp. 107-108. Use of Simocephalus Vetulus Embryos in the Teaching of Embryology, The, Gene W. Moser, February, pp. 96-98.

US Scientists Begin Cooperative Antarctic Research Program with Chilean Expedition, Editor, April,

p. 209.

Virus Reproduction, Editor, February, p. 76. "Vistas of Science," Editor, December, p. 512.

Water Pollution Conference, Editor, May, p. 275. Whack That Quack-Report of a Classroom Project that Involved the Community, Arlena E. Seneca, March. pp. 147-150.

Why Follow the Leader? Leonard G. Scheel, May,

pp. 276-278.

Women in Biology Leaflet, Editor, December, p. 512. Writing Articles for Publication, John Breukelman, October, pp. 359-364.

BOOK REVIEWS

Advances in Small Animal Practice, Vol. 11, Bruce V. Jones, October, p. 375. Aids to Histology, G. H. Bourne, February, p. 110.

American Arbacia and Other Sea Urchins, December, p. 528.

Anatomy of Plants, P. Font, April, p. 237.

Animal Diversity, Earl D. Hanson, December, p. 527. Animal Growth and Development, Maurice Sussman, January, p. 48.

Animal Husbandry Heresies, Allan Fraser, October,

Animal Physiology, Knut Schmidt-Nielsen, January, pp. 47-48.

Ants, William Morton Wheeler, December, p. 528. Apical Meristems, F. A. L. Clowes, December, p. 527.

Approach to Natural Science, Brehaut, Dawson, Grimsdell, Paul, and Skull, May, pp. 301-302.

Are School Teachers Illiberally Educated? Earl J. McGrath and Charles H. Russell, November, p.

540

Aspects of the Origin of Life, M. Florkin, May, p.

Atlas of Plant Morphology, Portfolio I: Photo Micrographs of Root, Stem and Leaf, Emma L. Fisk and William F. Millington, October, p. 370.

Atoms Within Us, Ernest Borek, December, pp. 533-

Attitudes of Liberal Arts Faculty Members Toward Liberal and Professional Education, Paul L. Dressel and Margaret F. Lorimer, November, p. 461.

Bailliere's Atlas of Female Anatomy, Katherine F. Armstrong and Douglas J. Kidd, April, p. 234. Basic Concepts and Experiments in Microbiology,

Delbert E. Schoenhard, October, p. 378.

Behavior Genetics, John L. Fuller and W. Robert Thompson, February, p. 112.

Biochemistry of Plants and Animals, Mallette, Althouse, and Clagetl, May, p. 301.

Biological and Chemical Control of Plant and Animal Pests, L. P. Reitz, Ed., January, p. 47.

Biologist's Handbook of Pronunciation, The, Edmund C. Jaeger, November, p. 461. Biology, A Basic Science, Elwood D. Heiss and

Richard H. Lape, October, p. 366.

Biology Investigations, Teacher's Edition, James H. Otto, Sam S. Blanc, Albert Fowler, March, p. 168. Biology of Weeds, John L. Harper, February, p. 109. Biology Through Microbes-A Laboratory Guide, Alfred S. Sussman, December, p. 531.

Biotic World and Man, Lorus Milne and Margery Milne, January, p. 46.

Bird Portraits in Color, Thomas Sadler Roberts, October, p. 375.

Book About Bees, Edwin Way Teale, January, p.

Carcinogenesis By Ultraviolet Light, Harold F. Blum, December, p. 534.

Cells and Societies, J. T. Bonner, December, p. 525. Cellular Slime Molds, J. T. Bonner, December, p.

Classic Papers in Genetics, James A. Peters, January, p. 49.

Classics in Biology, Sir S. Zuckerman, May, pp. 300-1. College Zoology, Robert W. Hegner and Karl A. Stiles, October, p. 371.

Communication Among Social Bees, Martin Lindauer, October, p. 373.

Comparative Effects of Radiation, Burton, Smith, Magee, May, p. 296.

Concepts of Medicine, Brandon Lush, December, pp. 530-531.

Cork and the Cork Tree, Giles B. Cooke, October, p. 371.

Critical Index of Films and Filmstrips in Conservation, A, Conservation Foundation, November, p. 459.

Culture Methods for Invertebrate Animals, Frank E. Lutz, Paul L. Welch, Paul S. Galtsoff, and James G. Neadham, October, p. 372.

Cutaneous Innervation, Advances in Biology of Skin, William Montagna, December, p. 530.

Developing Cell Systems and Their Control, Dorothea

Rudnick, May, p. 299.

DNA Model Kit, Van R. Potter, October, p. 379. Doubleday Pictorial Library of Nature-Earth, Plants, Animals, James Fisher, Sir Julian Huxley, Sir Gerald Barry, and J. Bronowski, October, p. 369.

Ecology of Inland Waters and Estuaries, George K. Reid, March, p. 169.

Educators Guide to Free Science Materials, Mary Horkheimer Saterstrom and John W. Renner, Eds, November, pp. 459-460.

Effective Reading in Science, David L. Shepherd, November, p. 459.

Electron Microscopy in Anatomy, J. D. Boyd, F. R. Johnson, and J. D. Lever, Eds., December, p. 531. Elementary Human Physiology, Terence A. Rogers,

October, p. 380. Elementary Human Physiology, Laboratory and Demonstration Manual, A. B. Taylor and Frederick Sargent, December, p. 529.

Elementary Physiology-A Laboratory Guide, Oscar E. Tauber, Robert E. Haupt, and Delma E. Harding, December, p. 529.

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Ed., December, p. 526 Encyclopedia of Microscopy, George L. Clark, December, p. 532.

Energies of the Universe, Eugene Fritz, May, p. 302. Evolution: Process and Product, Edward O. Dodson, January, p. 49.

Experimental Biochemistry, A Laboratory Manual, Gerald Litwock, May, p. 301.

Fellowships in the Arts and Sciences, 1961-1962, Michael Edmind Schiltz, November, p. 461. Fetal Pig Manual, C. A. Leone and P. W. Ogilvie,

October, p. 375.

Field List of Birds of the Detroit-Winds or Region, Ralph A. O'Reilly, Jr., Neil T. Kelly, Alice H. Kelly, March, p. 169.

Filmstrips (Geology and Soil), Marian Ray, May, pp. 296-297.

Forest and the Sea, Marston Bates, January, p. 49. Forest and Shade Tree Entomology, Roger T. Anderson, May, p. 297

Free and Inexpensive Educational Aids, Thomas J. Pepe, November, p. 460.

Functional Anatomy-Mammalian and Comparative, W. James Leach, October, p. 374.

Fundamentals of Plant Physiology, James F. Ferry and Henry S. Ward, December, pp. 526-527. Future of Man, P. B. Medawar, March, p. 170.

Galapagos, Irenaus Eibl-Eibesfeldt, May, p. 297. General Biology, Willis H. Johnson, Richard A. Laubengayer, and Louis DeLanney, December, pp. 525-526.

General Biology, William Taylor and Richard J. Weber, October, p. 367.

General Biology, Laboratory Guide, A. Paul Davison, February, p. 109.

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Grasses of Burma, Ceylon, India, and Pakistan, N. L. Bor, May, pp. 297-298.

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Gray's Anatomy, December, p. 530. Growth, Development and Pattern, N. J. Berrill, December, p. 534.

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Necturus, and the Cat, May, p. 297.
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Murice J. Gerstein, May, p. 299.

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Kenneth, November, p. 460. Herbicides and the Soil, E. K. Woodford and G. R.

Sager, May, p. 298. Herring Gull's World, Niko Tinbergen, April, p. 236. History of Life Science, Eldon J. Gardner, April, pp. 237-239.

History of Science Cases For High School, Case I, The Sexuality of Plants, Case II, Frogs and Batteries, Case IX, The Cells of Life, Leo E. Klopfer, October, p. 368.

How Life Began, Irving Adler, December, p. 533. How to Know the American Marine Shells, R. Tucker Abbott, October, p. 373.

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October, p. 373. Insects Close Up, Edward S. Ross, October, p. 373. Integrated Principles of Zoology, Cleveland P. Hick-

man, October, p. 371. Intelligent Man's Guide to Science, Vol. 1, The Physical Sciences, Vol. 2, The Biological Sciences,

Isaac Asimov, October, p. 368. Introduction to Animal Biology, Braugart and Bud-

deke, January, p. 48. Introduction to Parasitology, Asa C. Chandler and Clark P. Read, October, p. 378.

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October, p. 379. Invertebrate Zoology Laboratory Workbook, D. Elden Beck and Lee Braithwaite, October, p. 372.

Kranz Manual of Kinesiology, Clem W. Thomson, December, p. 530.

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Laboratories in the Classroom, November, p. 459. Laboratory Exercise in Genetics, Howard A. Royle, April, p. 235.

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Laboratory Manual for Dairy Microbiology, E. M. Foster and W. C. Frazier, December, pp. 531-532. Laboratory Manual for General Biology, Willis H. Johnson, Richard A. Laubengayer, and Louis E. DeLanney, December, p. 526.

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Laboratory Manual in General Biology, Lorus Milne

and Margery Milne, January, p. 46.

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A. Schanche, March, p. 171.

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Microbiology, Philip L. Carpenter, October, p. 378. Microtechnique, A Student's Guide to Slide-Making, Arthur W. Jones and John M. Carpenter, October,

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Mushrooms of the Great Smokies, L. R. Hesler, January, pp. 46-47.

Natural Resource Use in Our Economy, William H. Stead, February, p. 113.

New Developments in High School Science Teaching, National Science Teachers Association, November, p. 457.

Nucleic Acid Outlines, Vol. I, Van R. Potter, October, p. 379.

Ocean of Air, David I. Blumenstock, May, p. 302. Oncogenic Viruses, Ludwik Gross, December, pp. 532-533.

Parasitology: The Biology of Animal Parasites, Elmer R. and Glenn A. Noble, October, p. 377,

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Physiology of Work and Play, Sarah R. Riedman,

October, p. 381. Phytoplankton of Chesapeake Bay, Ruth E. Griffith, December, p. 527.

Planning for Excellence in High School Science, National Science Teachers Association, November,

Plant Community, Herbert C. Hanson and Ethan

D. Churchill, October, p. 370. Plant Kingdom, Harold C. Bold, February, p. 110. Playing With Words, Joseph T. Shipley, November, p. 463.

Policies for Science Education, Frederick L. Fitzpatrick, November, pp. 456-457.

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Principles of Animal Taxonomy, George Gaylord Simpson, October, p. 375.

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Principles of Modern Biology, Douglas Marsland,

October, p. 366.

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Principles of Zoology, A Laboratory Manual, Philip A. Buscemi, Jean Lauber, Stewart E. Schell, April,

Proceedings of the Indiana Academy of Science for 1959, Richard A. Laubengayer, Ed., November, p.

Projessional Manpower and Education in Communist China, Leo A. Orleans, November, pp. 461-462. Progress in Biophysics and Biophysical Chemistry,

J. A. Butler and B. Katz, May, p. 301.

Project Ideas for Young Scientists, John K. Taylor, Phoebe Knipling, and Falconer Smith, November, p. 456.

Quality Science for Secondary Schools, National Science Teacher's Association, November, p. 458. Quantitative Bacterial Physiology Laboratory Experiments, Michael J. Pelczar, Jr., P. Arne Hanse, and Walter A. Konetzka, October, p. 379.

Quantity and Quality of College Teachers, Earl J. McGrath, November, p. 461.

Scientific American Book of Projects for the Amateur Scientist, The, C. L. Stong, November, pp. 455-456.

Self Organizing Systems, Marshall C. Yoirts and

Scott Cameron, May, p. 300. 101 Simple Experiments With Insects, H. Kalmus, October, p. 373.

Soviet Education Programs, W. K. Medlin, C. B. Lindquist, and M. L. Schmitt, November, pp.

Space Biology—The Human Factors in Space Flight, J. S. Hanrahan and David Bushnell, April, p. 234. Spores II, H. Orin Halvorson, Ed., December, p. 532. STAR '60, Selected Papers on Science Teaching, Abraham Raskin, Editor, November, pp. 457-458. Starling's Human Physiology, Sir Charles Lovatt Evans, October, p. 381.

Structure and Function of the Body, Catherine Parker Anthony, January, p. 49.

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Studies in Paleobotany, Henry N. Andrews, Jr., May, pp. 298-299.

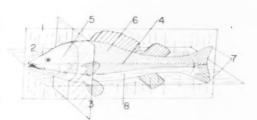
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Viruses and the Nature of Life, Wendell M. Stanley and Evans G. Valens, October, p. 379.

Weed Control Handbook, E. K. Woodford, April.

p. 237. Wellsprings of Life, Isaac Asimov, October, p. 369. Western Butterflies, Arthur C. Smith, October, p. 374.

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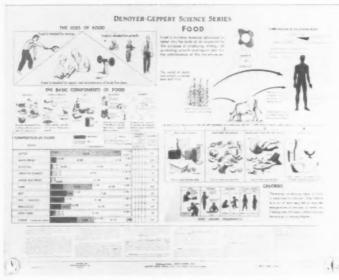
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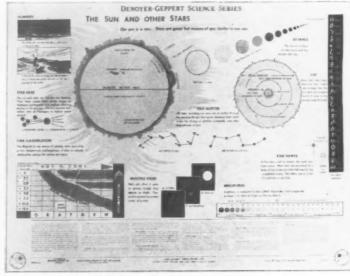
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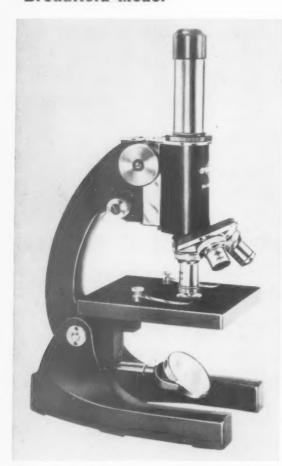
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THE LAWS OF OPTICS HOLD For a begin-FOR STUDENT MODELS TOO. any enlarged image seen through the microscope image seen through the microscope will appear exciting. But isn't it just as important to see a correct image? A true picture? Magnification without resolution is empty. . the image appears blurred and details are fringed with diffraction lines in much the same way as a faulty TV picture. That's why UNITRON doesn't ofer a 'student series' of objectives which, though named to imply "achromatic", still let color and aberrations in through the back door. All UNITRON Student Microscopes are equipped with the same professional-type objectives supplied on our more expensive medical models. Because our high-dry 40X objectives and condensers each have a medical models. Because our high-dry 40X objectives and condensers each have a numerical aperture of 0.65, the student can enjoy the same quality image at 400X or 600X that the medical student sees through his more expensive instrument.

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SOMETHING NEW HAS BEEN ADDED. All UNITRON Student Microscopes now have built-in focusing stops HAS BEEN ADDED. Microscopium stops built-in focusing stops that prevent accidental contact between the that prevent accidental contact Detween the stop of the sto that prevent accidental contact between the objective and specimen slide. This reduces repair costs for objectives and prevents slide breakage. Without the stop, it is easy for beginning students to pass through the critical point of focus, not even realize it, and ram the objective into the slide. The new stop also saves time and temper by automatically placing the image in approximate focus. Student guesswork is eliminated.

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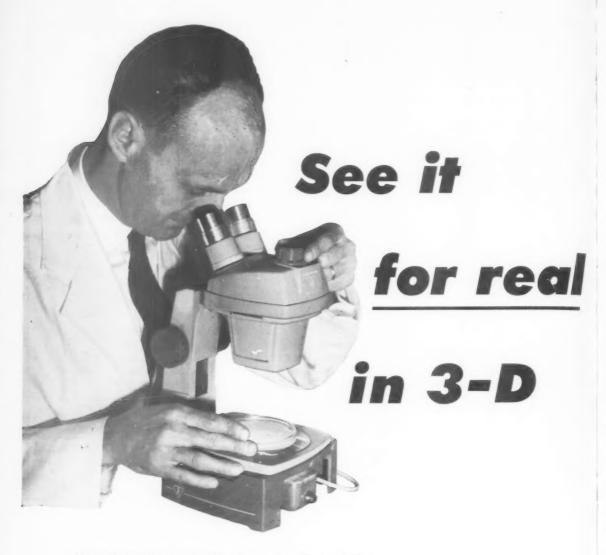
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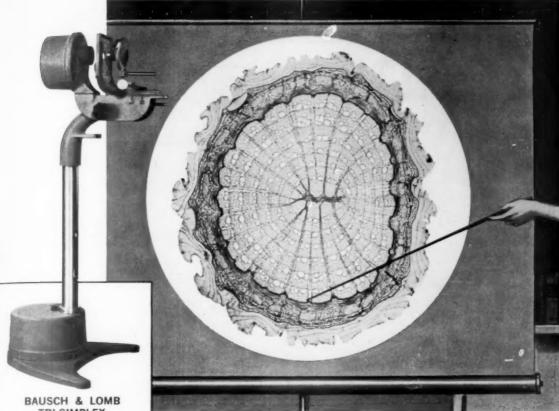
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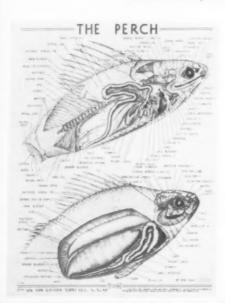
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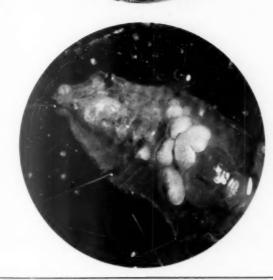
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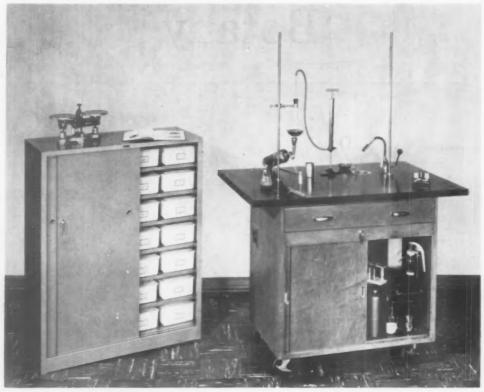


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